FORMULATION AND EVALUATION OF ETORICOXIB NIOSOMAL TOPICAL GEL

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CERTIFICATE

This is to certify that the dissertation entitled**, "Formulation and Evaluation of Etoricoxib Niosomal Topical Gel"** was done by **Mr.D.Rajiv Gandhi** in the Department of Pharmaceutics, Madurai Medical College, Madurai – 20, in partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmaceutics, is a bonafide work carried out by him, under the guidance and supervision of **Mr.A.Abdul Hasan Sathali**, **M.Pharm.,(Ph.D).,** Professor and Head, in the Department of Pharmaceutics, during the academic year 2010 – 2012.

This dissertation is forwarded to the Controller of Examinations, The Tamilnadu Dr. M.G.R. Medical University, Chennai.

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This is to certify that the Dissertation entitled **"Formulation and Evaluation of Etoricoxib Niosomal Topical Gel"** submitted by **Mr. D. Rajiv Gandhi** in partial fulfillment of the requirement for the degree of **Master of Pharmacy** in **Pharmaceutics** is a bonafide work carried out by him, under my guidance and supervision during the academic year 2010 – 2012 in the Department of Pharmaceutics, Madurai Medical College, Madurai-20.

I wish him success in all his endeavors.

Place: Madurai

Date: **(Prof.A.Abdul Hasan Sathali)**

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ABBREVIATION

Abbreviation	Expansion
nm	Nanometer
λ_{max}	Wavelength with maximum absorbance
%	Percentage
IR	Infra red spectra
H	Hour
&	And
Rpm	Revolutions per minute
GIT	Gastrointestinal tract
BCS	Biopharmaceutical classification
MLV	Multilamellar vesicles
SUL	Small unilamellar vesicles
LUV	Large unilamellar vesicles
OLV	Oligo lamellar vesicles
CMC	Critical micellar concentration
CMT	Critical micellar temperature
MM	Mixed micelles
NDDS	New drug delivery system
EDTA	Ethylene diamine tertaacetic acid
NSAIDS	Non steroidal anti-inflammatory drugs
TC	Phase transition temperature
DCP	Dicetyl phosphate
STR	Stearylamine
CPP	Critical packing parameter
REV	Reverse phase evaporation
OM	Optical microscopy
PCS	Photon correlation spectroscopy
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy
UV	Ultra violet spectroscopy
DSC	Differential scanning calorimetry
PG	Prostaglandins
RH	Relative humidity
PBS	Phosphate buffer saline
ANOVA	One way analysis of variance

CHAPTER-I

INTRODUCTION

COLLOIDAL DRUG DELIVERY SYSTEMS

During the past decade, advanced drug delivery research and development has surged ahead of others in formulation research. The emergence of novel technology and the growing capabilities of proteomics, genomics and combinatorial chemistry have provided scientists with new technologies.

Commonly accepted criteria of advanced drug delivery system include

a) Maximal drug bioavailability
b) Tissue targeting
c) Controlled release kinetics
d) Minimal immune response
e) Ease of administration for better patient compliance
f) Ability to deliver different drugs such as lipophilic, amphiphiles and biomolecules.
g) Enhanced permeability of poorly permeable drugs.
h) Avoidance of side effects by reducing the dose and frequency of drug intake (Chein *et al,* 2005).

Therapeutic efficacy of drug depends on four fundamental pathways of drug transport and modification within the body

Absorption into the plasma from the administration site.

Distribution between plasma and tissues.

Metabolism within the tissues.

Elimination from the body.

Absorption rate depends on many factors such as hydrophobicity, chemical environment, particle size, crystallinity, blood flow, absorptive surface area and residence time at absorptive surface (Delie *et al*, 2005).

Drug distribution largely depends on blood flow, capillary permeability such as in BBB, ligand binding and hydrophobicity.

Drug metabolism and elimination also depend on above mentioned properties. The drug delivery system can greatly impact each pathway, and, therefore, the delivery system is a critical design component in pharmaceutical sciences.

Lipophiles (or) poorly water soluble drug, perform pivotal roles in numerous biological processes. Many leading small molecules of drugs are lipophilic. Anticancer drug including piposulfan, etoposide, camptothecin and paclitaxel are lipophilic. Antifungal drugs such as amphotericin-B, fluconazoloe, itraconazole are lipophilic. Key antioxidants such as vitamin A, vitamin E, retinol, lycophene and β-carotene also are lipophilic. These lipophiles must be formulated and delivered in a safe, efficacious and cost effective manner. Lipophile delivery has long been a challenge in pharmaceutical sciences.

Main reasons for failure in therapy include

- Insufficient drug concentration at the site of action due to poor absorption, rapid metabolism, and elimination of drug distribution to other tissues combined with high drug toxicity.
- Poor drug solubility which excludes I.V. injection of aqueous drug solution.
- High fluctuation of plasma levels due to unpredictable bioavailability after per oral administration, including the influence of food on plasma levels.

A promising strategy to overcome these problems involves the development of suitable drug colloidal carrier system (Hussain *et al,* 1999).

Need of colloidal carrier systems

Colloidal carriers are promising systems to fulfill the requirements of a poorly aqueous soluble drug. Nanosized carriers are treated as hopeful means to increase the solubility and the bioavailability of poorly water soluble – active ingredients belonging to the classes II and IV in the biopharmaceutical classification system [BCS], (Martins *et al,* 2007, Sachan *et al,* 2006).

The common characteristic of all colloidal carriers is the sub-micron particle size. Corresponding to the broad diversity of colloidal carriers, the possible administration routes vary as follows

- Dermal
- Peroral
- Parenteral
- Ocular
- Pulmonary

For I.V. administration, to avoid embolism in blood vessels, no particles above 5 μm and only few particles between one to five micrometers are accepted. Solid particulate systems are limited to either the subcutaneous or intramuscular routes of administration; intravenous administration may result in vaso occlusion.

Focusing on the biofate of lipid – containing drug carriers after per oral administration, short chain and medium chain liquid lipids are known to be easily hydrolyzed and to be readily absorbed in the gastrointestinal tract [GIT]. Crystalline lipids are poorly attacked by lipases and very long chains (From C18 up) in solid state are poorly absorbed (Basu *et al*, 2003).

Nanosizing of the bulk material may lead to dramatic changes of the physical properties of the substance. There may be depression of the melting point which results in the existence of super cooled melts. Nanometric systems have to fulfill the requirements of drug delivery systems mentioned above. They should be free from aggregation/ coalescence tendency. Incorporation of sensitive drug molecule in some carrier matrices is claimed as a protection against enzymatic degradation, hydrolysis or photolysis. Despite their small size, colloidal carriers can provide controlled drug release.

Colloidal drug carrier systems

1.1Liposomes

Liposomes consist of one or more lipid bilayers of amphiphilic lipids (phospholipids, cholesterol, glycolipids). The lipophilic moieties of the bilayer face each other and create an inner hydrophobic environment in the membrane. Lipophilic or amphiphilic drugs can be associated with non polar parts of lipid bilayers if they fit in size and geometry. The hydrophilic molecular head groups face the outer water phase and the inner aqueous core of the vesicles. Water soluble compounds can be included within the aqueous compartments (Bawarski *et al*, 2008, Biju *et al*, 2006).

Liposomes are classified as large multilamellar liposomes (MLV), large unilameller vesicles(LUV), small unilameller vesicles (SUV), oligolameller vesicles (OLV) and multivesicular (MVU) systems, depending on the their size, the number of bilayers and the existence of inner vesicles in a vesicle. The size of liposomes varies from 20nm to few micrometers with lipid membranes approximately 5nm.

Some of the marketed products are

- Ambisome ^{TM} -parenteral
- Daunoxome ^{TM}-parenteral
- Prvaryl ^{TM}- Lipogel- Topical administration.

1.2 Niosomes

Niosomes are novel drug delivery systems, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agent and hence the name niosomes. The niosomes are very small rather microscopic in size. Their size lies in the nano metric scale. Although structurally similar to liposomes, they offer several advantages over them. Niosomes can greatly increase transdermal drug delivery and also can be used in targeted drug delivery, and thus increased study in these structures can provide new methods for drug delivery.

1.3 Nanocapsules

Oil containing nanocapsules differ from nanoemulsions in providing a barrier made from polymer between the core and the surrounding environment. Reports on nanoparticles with aqueous cores in an aqueous outer phase are published. For the preparation of nanocapsules the way the solvent displacement and interfacial polymerization are achieved are individualized (Kreuter *et al*, 2007, Magalhaes *et al*, 1995).

According to the liphophilicity of the capsule content, hydrophilic and lipophilic drugs can be dissolved additionally. The polymeric particle surface may serve as compartment. Encapsulation may decrease the toxicity of drugs after peroral or parenteral application. Encapsulation saves sensitive drugs from rapid degradation, reduce the interactions with

reticuloendothetial system (RES) and alter body distribution the surface of nanocapsules can be modified by materials such as surfactants.

1.4 Nanosuspensions

Nanosuspensions are saturated solutions. They represent the simplest colloidal carriers with respect to composition. The drug payload amounts to nearly 100%. In an aqueous environment, the drug is pearl milled, precipitated or high pressure homogenized to a particle distribution mostly below one micrometer. Despite the use of tensides, particle growth up to micrometric crystals may occur when the drug molecules of small particles dissolve in the outer environment and precipitate later on the surface of larger particles (Patravale *et al*, 2004, Constartinides *et al*, 2008).

Due to tremendous interface area between drug and environment, solubilization velocity of the drug is increased according to the Noyes Whitney equation. Attention has to be paid on drugs with small safety margins where burst release has to be avoided. But controlled release and reproducible blood levels are not easily achievable because as a release controlling barrier only the tenside layer may serve in the nanosuspension. Suspensions of crystals in the μm range are already established in the market (eg: Predigaleu ^{TM}). The only two registered Nanosuspensions are Repamune ^{TM} and Emend ^{TM} for immediate delivery.

1.5 ***Mixed micelles***

As long – chain phospholipids are known to form bilayers when dispersed in water, the preferred phase of short-chain analogues is the micellar phase. In general ampholytic molecules, which are able to decrease the surface tension of a solvent, arrange in micelles, as Tween ^{TM} or sodium dodecyl sulfate. A micellar solution is a thermodynamically stable system formed spontaneously in water or in organic solvents. The small colloidal aggregates (micelles) are in

rapid thermodynamic equilibrium with a measurable concentration of monomer in micellar solutions. The micelle solubilizes large molecules in any zone of the micelle volume, but the penetration into the micelle depends on the inner space of the micelle, on the hydrophobicity of the drug and on the charge of the incorporated molecule. The interaction between micelles and lipophilic drugs leads to the formation of mixed micelles (MM), often called as swollen micelles. The addition of salt or alcohol can vary the degree of penetration into the micelle. In mixed micelles, the mobility of the micellar phase was found to decrease due to incorporated molecules. Considerably swollen micelles are larger than the analogous "free micelles" because solubilization may result mostly from the increase in micellar site (Bawarski *et al*, 2005, Torchilin *et al*, 2006).

CHAPTER-II

A REVIEW ON VESICULAR SYSTEM

INTRODUCTION

Many drugs, particularly chemotherapeutic agents, have narrow therapeutic window. Their clinical use is limited and compromised by dose limiting toxic effect. The therapeutic effectiveness of the existing drugs is improved by formulating them in an advantageous way. In the past few decades, considerable attention has been focused on the development of new drug delivery systems (NDDS). The NDDS should deliver the drug at a rate directed by the needs of the body, over a predetermined period of treatment and it should channel the active entity to the site of action. Conventional dosage forms and prolonged release dosage forms are unable to meet these requirements.

In recent years, vesicles have become the carriers of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques and genetic engineering.

Vesicles can play a major role in modeling biological membranes and in the transport and targeting of active agents (Umeda *et al*, 1997).

Conventional chemotherapy for the treatment of intracellular infections is not effective, due to limited permeation of drugs into cells. This can be overcome by use of vesicular drug delivery systems. Encapsulation of a drug in vesicular structures can prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved (Todd *et al*, 1982).

The phagocytic uptake of the systemic delivery of the drug-loaded vesicular delivery system provides an efficient method for delivery of drug directly to the site of infection, leading to reduction of drug toxicity with no adverse effects.

Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. Vesicles can incorporate both hydrophilic and lipophilic drugs.

Vesicular drug delivery systems delay drug elimination of rapidly metabolizable drugs, and function as sustained release systems. This system solves the problems of drug insolubility, instability, and rapid degradation.

Advantages of vesicular systems:

- Efficient method for delivery of drug directly to the site of infection.
- Reduction of drug toxicity with no adverse effects.
- Reduces the cost of the therapy by improved bioavailability of the medication,
- Incorporate both hydrophilic and lipophilic drugs.
- Delay drug elimination of rapidly meatbolizable drugs
- Function as sustained release systems.
- Solves the problems of drug insolubility, instability, and rapid degradation.

2.1 TYPES OF VESICULAR SYSTEMS

Various types of vesicular systems are as follows

- Liposomes
- Niosomes
- Transferosomes
- Pharmacosomes

- Enzymosomes
- Virosomes
- Ufasomes
- Cryptosomes
- Emulsomes
- Discomes
- Aquasomes
- Ethosomes
- Genosomes
- Photosomes
- Erythrosomes
- Hemosomes
- Proteosomes
- Vesosomes
- Archaeosomes
- Apsasomes
- Colloidosomes
- Cubasomes

2.2 LIPOSOMES

Liposomes are simple microscopic vesicles in which lipid bilayer structures are present with an aqueous volume entirely enclosed by a membrane composed of lipid molecule. There are a number of components present in liposomes, with phospholipid and cholesterol being the main

ingredients. The type of phospholipids includes phosphoglycerides and sphingolipids together with their hydrolysis products.

2.2.1Liposomes Preparation Methods

A) Multilamellar Liposomes (MLV)

i) Lipid Hydration Method

(a) This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities. MLV are simple to prepare by this method and a variety of substances can be encapsulated in these liposomes. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is not normal (Bengham *et al*, 1974).

(b) MLVs with high encapsulation efficiency can be prepared by hydrating the lipids in the presence of an immiscible organic solvent (petroleum ether, diethyl ether). The contents are emulsified by vigorous vortexing or sonication. The organic solvent is removed by passing a stream of nitrogen gas over the mixture. MLVs are formed immediately in the aqueous phase after the removal of organic solvent. The main drawback of this method is the exposure of the materials to be encapsulated to organic solvent and to sonication (Papahadjopoulos *et al*, 1978).

ii) Solvent Spherule Method

A method for the preparation of MLVs of homogeneous size distribution was proposed by Kim *et al*, 1985. The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath (Kim *et al*, 1985).

B) Small Unilamellar Liposomes (SUV)

i) Sonication Method

Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV (Oezden *et al*, 1991).

(ii) French Pressure Cell Method

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50mL maximum), (Hamilton *et al*, 1984).

C) Large Unilamellar Liposomes (LUV)

They have high internal volume/encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

I. SOLVENT INJECTION METHODS

a) *Ether Infusion Method*

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is slowly injected in to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature (Sehieren *et al*, 1978).

b) Ethanol Injection Method

A lipid solution of ethanol is rapidly injected in to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of interaction of various biologically active macromolecules in presence of even low amounts of ethanol (Batzri *et al*, 1973).

II. DETERGENT REMOVAL METHODS

The detergents at their critical micellar concentration have been used to solubilise lipids. As the detergent is removed the micelles become progressively richer in phospholipid and finally combine to form LUVs. The detergents were removed by dialysis. The advantages of detergent dialysis method are excellent reproducibility and production of liposome populations which are homogenous in size. The main drawback of the method is the retention of traces of detergent(s) within the liposomes. A commercial device called LIPOPREP which is a version of dialysis system is available for the removal of detergents. Other techniques have been used for the removal of detergents: (a) by using Gel Chromatography involving a column of Sephadex G-25,

(b) by adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2. (c) By binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads (Philippot *et al*, 1985).

iii. Reverse Phase Evaporation Method

First water in oil emulsion is formed by brief sonication of a two phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. (Handa *et al*, 1984).

iv. Calcium Induced Fusion Method

This method is used to prepare LUV from acidic phospholipids; calcium addition to SUV induces fusion and results in the formation of multilamellar structures in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs. Here macromolecules can be encapsulated under gentle conditions and the chief disadvantage of this method is that LUVs can only be obtained from acidic phospholipids (Mayhew *et al*, 1984).

D. Giant Liposomes

The procedure for the formation of giant liposomes involves the dialysis, of a methanol is solution of phosphatidylcholine in the presence of methylglucoside detergent against an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm (Oku *et al*, 1983).

E. Multivesicular Liposomes

The water in oil emulsion was converted to organic solvent spherules by the addition of the emulsion to solution. The evaporation of organic solvent resulted in the formation of

multivesicular product. The diameter of liposomes ranges from 5.6 to 29 pm. The materials which can be encapsulated include glucose, EDTA, human DNA. These liposomes have very high encapsulation efficiency (up to 89%), (Kim *et al*, 1983).

Advantages and Disadvantages

- Liposomes as a potential delivery system for the oral administration of insulin have been extensively studied (Patel *et al*, 1982, Hashimoto *et al*, 1979, Derycke *et al*, 2004, Allen *et al*, 1978).
- The liposomes have protective effects against proteolytic digestive enzymes like pepsin and pancreatin and they can increase the intestinal uptake of macromolecules.
- Long-circulating and actively targeting liposomes, stand a better chance as truly tumor tropic carriers of photosensitizers.
- Liposomal drug delivery systems provide protection of the drug, controlled release of the active moiety along with the targeted delivery, and cellular uptake via endocytosis.
- Liposomes also pose certain problems like degradation by hydrolysis, oxidation, sedimentation, leaching of drug and aggregation or fusion during storage.
- Liposome stability is increased by efficient formulation and lyophilization.
- Formulation involves the selection of the appropriate lipid composition and concentration of the bilayer, in addition to the aqueous phase ingredients, such as buffer, antioxidants, metal, chelators, and cryoprotectant. Charge-inducing lipids, such as phosphatidylglyceride are incorporated into the liposome bilayer to decrease fusion, while cholesterol and sphingomyelin can be incorporated in formulations, in order to decrease the permeability and leakage of encapsulated drugs. Buffers decrease hydrolysis.

- Addition of antioxidants can decrease the oxidation. Freeze-dried liposome formulations should incorporate a lipo protectant-like non-reducing disaccharide such as trehalose or sucrose. Difficulties are experienced in sterilization and large-scale production of liposomes.
- The cost and purity of phospholipid is another limiting factor. They are suitable for parenteral administration but oral administration is not possible because of inability of liposomes to survive to the action of bile salts and phospholipids.

2.3 NIOSOMES

Niosomes are novel drug delivery systems in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agent and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the nano metric scale. Although structurally similar to liposomes, they offer several advantages over them. Niosomes have been shown to greatly increase transdermal drug delivery and also can be used in targeted drug delivery. These structures can provide new methods for drug delivery.

2.4 TRANSFEROSOMES

- Liposomes and niosomes are the vesicular carrier systems of drug delivery (Arun *et al*, 2005, Jain *et al*, 1998).
- For transdermal delivery a new type of carrier system called “transfersomes” was introduced for the delivery of number of low and high molecular weight drugs. It consists of both hydrophilic and hydrophobic properties with high deformability gives better penetration of intact vesicles.
- Transfersomes may be described as lipid droplets of such deformability that permits its easy penetration through the pores much smaller than the droplets size. They protect the encapsulated drug from metabolic degradation.

- In thermodynamic terms this typically corresponds to an aggregate in the quasi-metastable state, which facilitates the formation of highly curved bilayers. From the composition point of view, transfersomes are a self adaptable and optimized mixed lipid aggregate. They act as depot, releasing their content slowly and gradually.
- Transfersomes have been developed in order to take advantage of phospholipid vesicles as transdermal drug carrier. These self optimized aggregates, with ultra flexible membrane, are able to deliver the drug reproducibly either into or through the skin, depending on the choice of administration or application, with high efficiency.
- These vesicular transfersomes are several orders of magnitude more elastic than the standard liposomes and thus well suited for the skin penetration. Transfersomes overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of stratum corneum. There is provision for this, because of the high vesicles deformability, which permits the entry due to mechanical stress of surroundings in a self adapting manner.
- Flexibility of transfersome membrane is achieved by mixing suitable surface active agents in proper ratios. The resulting flexibility of transfersomes membrane minimize the risk of vesicle rupture in the skin and allow transfersomes to follow the natural water gradient across the epidermis when applied under non occlusive condition.
- Transfersomes can penetrate the intact stratum corneum spontaneously either through intracellular route or transcellular route. The high and self-optimizing deformability of typical composite transferosmes membrane, which are adaptable to ambient stress allow the ultra deformable transfersomes to change its membrane composition locally and reversibly, when it is pressed against and attracted into narrow pore. Some of the limitations which have been encountered is that transferosmes are chemically unstable because of their

predisposition to oxidative degradation as well as purity of natural phospholipid is another criteria militating against adoption of transfersomes as drug delivery vehicles.

Application

- Transfersomes have been widely used as carrier for the transport of proteins and peptides.
- Delivery of insulin by transfersomes is the successful means of non invasive therapeutic use of such large molecular weight drugs on the skin.
- Transfersomes have also been used as a carrier for interferons.
- Transfersomes as drug delivery systems have the potential for providing controlled release of the administred drug and increasing the stability of labile drugs.
- Another most important application of transfersomes is transdermal immunization using transfersomes loaded with soluble protein like integral membrane protein, human serum albumin, and gap junction protein**.** This approach offers at least two advantages, first they are applicable without injection and second, they give rise to rather high titer and possibly, to relatively high IgG A levels.
- Transfersomes have also been used for the delivery of corticosteroids. Transfersomes improve the site specificity and overall drug safety of corticosteroid delivery into skin by optimizing the epicutaneously administered drug dose. Transfersomes based corticosteroids are biologically active at dose several times lower than the currently used formulation for the treatment of skin diseases.
- Application of anesthetics in the suspension of highly deformable vesicles, transfersomes, induces a topical anesthesia, under appropriate conditions, with less than 10 min. Maximum resulting pain insensitivity is nearly as strong (80%) as that of a comparable subcutaneous bolus injection, but the effect of transferosomal anesthetics last longer.

- Transferosmes have also been used for the topical analgesics, anaesthetics, NSAIDS and anti-cancer agents.

Limitations

1. Transfersomes are chemically unstable because of their predisposition to oxidative degradation,
2. Lack of purity of the natural phospholipids comes in the way of adoption of transfersomes as drug delivery vehicles.
3. Transfersome formulations are expensive.

2.5 PHARMACOSOMES

- The limitations of transfersomes can be overcome by the "pharmacosome" approach. The prodrug conjoins hydrophilic and lipophilic properties, and therefore acquires amphiphilic characters, and similar to other vesicle forming components, was found to reduce interfacial tension, and at higher concentrations exhibits mesomorphic behavior (Goymann et al, 1991).
- These are defined as colloidal dispersions of drugs covalently bound to lipids, and may exist as ultrafine vesicular, micellar or hexagonal aggregates. Problems of drug incorporation, leakage from the carrier or insufficient shelf life can be avoided by the pharmacosome approach (Zhang *et al*, 2001).
- The idea for the development of the vesicular pharmacosome is based on surface and bulk interactions of lipids with drug. Any drug possessing an active hydrogen atom (-COOH, -OH, -NH2, etc.) can be esterified to the lipid, with or without spacer chain. Synthesis of such a compound may be guided in such a way that strongly result in an amphiphilic compound, which will facilitate membrane, tissue, or cell wall transfer, in the organism (Vaizoglu *et al*, 1986).

The salient features of Pharmacosomes are

- Entrapment efficiency is not only high and predetermined, because drug itself in conjugation with lipids forms vesicles.
- Unlike liposomes, there is no need of following the tedious, time-consuming step for removing the free, unentrapped drug from the formulation.
- Since the drug is covalently linked, loss due to leakage of drug, does not take place. However, loss may occur by hydrolysis.
- Encaptured volume and drug-bilayer interactions do not influence entrapment efficiency.

- In pharmacosomes membrane fluidity depends upon the phase transition temperature of the drug lipid complex, but it does not affect release rate since the drug is covalently bound (Volkering *et al*, 1995).
- The drug is released from pharmacosome by hydrolysis (including enzymatic).
- Phospholipid transfer/exchange is reduced, and solubilization by HDL is low.
- The physicochemical stability of the pharmacosome depends upon the physicochemical properties of the drug lipid complex.
- Due to their amphiphilic behavior, such systems allow, after medication, a multiple transfer through the lipophilic membrane system or tissue, through cellular walls piggyback endocytosis and exocytosis.
- Following absorption, their degradation velocity into active drug molecule depends to a great extent on the size and functional groups of drug molecule, the chain length of the lipids, and the spacer. These can be varied relatively precisely for optimized in vivo pharmacokinetics.
- They can be given orally, topically, extra or intravascularly.

Method of Preparation

In general two methods have been employed to prepare pharmacosomes. They are

1. Hand-shaking method

2. Ether-injection method

Hand-shaking method

In the hand-shaking method, the dried film of the drug– lipid complex (with or without egg lecithin) is deposited in a round-bottom flask and upon hydration with aqueous medium, readily gives a vesicular suspension.

The ether injection method

An organic solution of the drug– lipid complex is injected slowly into the hot aqueous medium wherein the vesicles are readily formed. At low concentration the amphiphiles exists in the monomer state. Further increase in monomers may lead to variety of structures i.e., micelles of spherical or rod like or disc shaped type or cubic or hexagonal shape. Compared the effect of diglyceride prodrug on interfacial tension, with the effect produced by a standard detergent dodecylamine hydrochloride, and found similar effect on lowering of surface tension. Above the critical micelle concentration (CMC), the prodrug exhibits mesomorphic lyotropic behavior, and assembles in supramolecular structures (Steve *et al*, 1996, Taskintune *et al*, 1997).

2.6 COLLOIDSOMES

Colloidsomes are a novel class of microcapsules whose shell consists of coagulated or fused colloid particles at interface of emulsion droplets. The particles self assemble on the surface of droplets in order to minimize the total interfacial energy forming colloidsomes. Such structures were produced for first time by templating latex particles adsorbed on the surface of octanol-in-water emulsion drops and subsequent removal of oil fusing the particle monolayers.

POTENTIAL BENEFITS OF COLLOIDOSOMES

- Efficient encapsulation; size, permeability, mechanical strength and compatibility can be easily controlled.
- Control of the size allows flexibility in applications and choice of encapsulated materials.
- Control of compatibility allows encapsulation of sensitive ingredients, such as biomolecules and cells.

LIMITATIONS

A major problem in the colloidosome manufacture is the poor yield of particles. If the shell locking is inefficient then the colloidosomes simply coalesce and fall apart on transfer into water.

2.7 ETHOSOMES

Ethosomes are novel lipid carriers. Ethosomal systems are novel permeation enhancing lipid carriers embodying ethanol containing lipid vesicles with inter digitated fluid bilayers. Ethosomes have high encapsulation efficiency, skin deposition ability and depth of skin penetration for a wide range of molecules including lipophilic drugs. Whether for pharmaceutical purposes, gene therapy, vaccination or cellular transformations in biomedical research the delivery of molecules through the biological membrane has become a major focus of research in recent years for which ethosomes are well suited.

2.8 ENZYMOSOMES

Liposomal construct engineered to provide a mini bio environment in which enzymes are covalently immobilized or coupled to the surface of the liposomes. They are used for targeted delivery to tumor cells.

2.9 VIROSOMES

Liposomes spiked with virus glycoprotein, incorporated into the liposomal bilayers based on retro virus derived lipids. They are used for immunological adjuvants.

2.10 UFASOMES

Vesicles enclosed by fatty acids obtained from long chain fatty acids (oleic acid, linoleic acid) by mechanical agitation of evaporated films in the presence of buffer solutions. They are used for ligand mediated drug targeting.

2.11 CRYPTOSOMES

Lipid vesicles with a surface coat composed of PC and of suitable polyoxyethylene derivative of phosphatidylethanolamine. They are used for ligand mediated drug targeting.

2.12 EMULSOMES

Nanosize lipid particles (bioadhesive nano emulsion) consisted of microscopic lipid assembly with a polar core which contains water insoluble drugs in the solution form without requiring any surface active agent or co solvent. These fat cored lipid paticles are dispersed in an aqueous phase. Emulsome represents lipid based drug delivery of systems with wide range of therapeutic applications especially for parenteral delivery of poorly water soluble drugs.

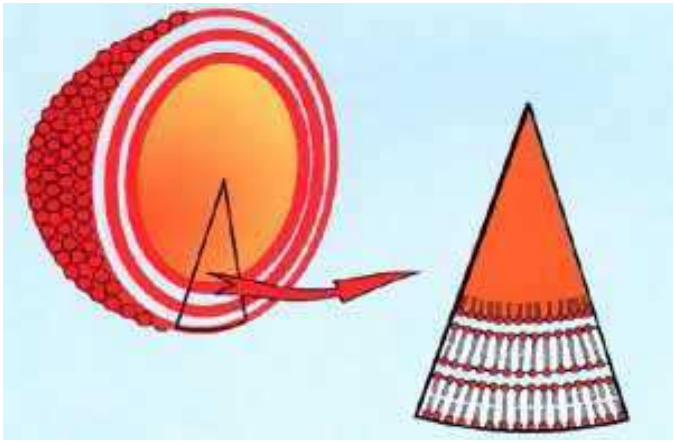


Figure 1. Diagrammatic structure of emulsome

2.13 DISCOMES

These are niosomes solubilized with non-ionic surfactant (Polyoxyethylene cetyl ether class, Solulan C_{24}). Discomes are large (12-60μm) structures and are capable of entrapping water-soluble solutes. They are used for ligand mediated drug targeting.

2.14 AQUASOMES

They are three layered self-assembly compositions with ceramic carbon nanocrystalline particulate core coated with glassy cellobiose. They are used for specific targeting, molecular shielding.

2.15 GENOSOMES

They are macromolecular complexes for functional gene transfer. They are used for cell specific gene transfer.

2.16 PHOTOSOMES

They are photolyase encapsulated liposomes. Which release the contents by photo-triggered charges in membrane permeability characteristics. They are used for photo dynamic therapy.

2.17 ERYTHROSOMES

Red blood cells offer a number of possibilities as drug carriers in controlled drug delivery systems. The release rate from erythrosomes, longevity and physical characteristics can be easily manipulated to alter the delivery mechanism. They are used in both site-directed and sustained-release systems. Liposomal systems in which chemically cross linked human erythrocyte cytoskeletons are used as a support on which lipid bilayer is coated. They are used for effective targeting of macromolecular drugs. Artificial red blood cells prepared by encapsulating hemoglobin by interfacial polymerization have been used as oxygen carriers.

2.18 HEMOSOMES

Haemoglobin containing liposomes engineered by immobilizing haemoglobin with a polymerisable phospholipid are called hemosomes. They are used for high capacity oxygen carrying system.

2.19 PROTEOSOMES

High molecular weight multi-subunit enzyme complexes with specific assembly pattern of enzymes are known as proteosomes. They are used for better catalytic activity turnover than non-associated enzymes.

2.20 VESOSOMES

Nested bilayer compartments in vitro via the “interdigitated” bilayer phase formed by adding ethanol to a variety of saturated phospholipids are called vesosomes. Multiple compartments of the vesosome give better protection to the contents in serum.

2.21 ARCHAEOSOMES

Archaeosomes are vesicles composed of glycerolipids of archaea with potent adjuvant activity.

2.22 APSASOMES

Ascorbyl palmitate vesicles are known as apasomes.

Ascorbyl palmitate (ASP) was explored as bilayer vesicle forming material. Vesicles prepared with amphiphiles having antioxidant property may have potential applications towards disorders implicated with reactive oxygen species. Ascorbic acid (vitamin-C) is a major antioxidant in human plasma as well as in and across cell membranes. It reduces α-tocopherol as well as peroxides and reactive oxygen species such as superoxide.

2.23 CUBOSOMES

Cubosomes consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. Self-assembled cubosomes as active drug delivery systems and they exhibit different internal cubic structure and composition with different drug-loading modalities. Cubosomes are nanoparticles whose size ranges from 10-500nm in diameter they appear like dots square shaped, slightly spherical.

CHAPTER-III

REVIEW ON NIOSOMES

Targeted delivery of anticancer and anti infective drugs is a challenging task with the use of novel drug delivery systems. Different novel approaches used for delivering drugs include liposomes, microspheres, nanotechnology, micro emulsions, antibody-loaded drug delivery, magnetic microcapsules, implantable pumps and niosomes. Niosomes are formations of vesicles by hydrating mixture of cholesterol and nonionic surfactants (Handjani *et al*, 1979). These vesicles are called niosomes. These are formed by self-assembly of non-ionic surfactants in aqueous media as spherical, unilamellar, multilamellar system and polyhedral structures in addition to inverse structures which appear only in non-aqueous solvent (Sternberg *et al*, 1995, Uchegbu and Florence 1995, Murdan *et al*, 1998). The process of vesicle formulation by self-assembly of nonionic surfactants is rarely spontaneous and usually requires some input of energy through physical agitation, extrusion or heat (Lasic *et al*, 1990).

Niosomes and liposomes are equiactive in drug delivery potential. Niosomes are preferred over liposomes because the former exhibit high chemical stability and economy (Hunter *et al*, 1988). Niosomes also exhibit special characteristics such as ease of handling and storage. Surfactant, forming niosomes are biodegradable, non-immunogenic and biocompatible.

3.1 ADVANTAGES OF NIOSOMES

- The niosomal drug delivery is a potential drug delivery method for controlled and targeted drug delivery.
- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non aqueous phase (Pranshu *et al*, 2011).

- The vesicle suspension is water–based vehicle and offer patient convenience.
- They are osmotically active and stable.
- Handling and storage requires no special conditions.
- They improve oral bioavailability of poorly absorbed drugs. They enhance skin penetration of drugs (Madhav *et al*, 2011).
- They can be made to reach the site of action by oral, parenteral as well as topical routes.
- The surfactants used are biodegradable, biocompatible and non-immunogenic.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells (Attaullah *et al*, 2011).
- Niosomes possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities (Kumar *et al*, 2011).
- The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, surface charge and concentration can control the vesicle characteristics.
- The vesicles may act as a depot, releasing the drug in a controlled manner.

3.2 FACTORS AFFECTING NIOSOME FORMULATION

Drug

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size. In polyoxyethylene glycol (PEG) coated vesicles; some

drug is entrapped in the long PEG chains, thus reducing the tendency to increase the size. The Hydrophilic lipophilic balance of the drug affects degree of entrapment (Raja *et al*, 1994).

Structure of surfactants

The geometry of vesicle is affected by structure of surfactant. On the basis of critical packing parameters of surfactants we can predicate geometry of vesicle to be formed. Critical packing parameters can be defined using following equation,

CPP (Critical Packing Parameters) = $CPP = \frac{V}{\ell_c . a_o}$

Where V = hydrophobic group volume,

ℓ_c = the critical hydrophobic group length,

a_o= the area of hydrophilic head group.

From the critical packing parameter value the type of miceller structure formed can be ascertained as given below,

If CPP < ½ then spherical are micelles formed.

If CPP < 1½ formation of bilayer micelles are formed.

If CPP > 1 inverted micelles are formed.

Amount and type of surfactant

The mean size of niosomes increases proportionally with increase in the HLB of surfactants like Span 85 (HLB 1.8) to Span 20 (HLB 8.6), because the surface free energy decreases with an **i**ncrease in hydrophobicity of surfactant. Bilayers of the vesicles are either in the so-called liquid state or in gel state, depending on the temperature, the type of lipid or surfactant and the presence of components such as cholesterol. In the gel state, alkyl chains are present in a well-ordered structure, and in the liquid state, the structure of the bilayers is more

disordered. The surfactants and lipids are characterized by the gel-liquid phase transition temperature (TC).Phase transition temperature (TC) of surfactant also effects entrapment efficiency for e.g. Span 60 has higher TC than other surfactant and provides better entrapment.

Cholesterol content and charge

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. The action of cholesterol is two fold on the one hand, cholesterol increases the chain order of liquid-state bilayers and on the other, cholesterol decreases the chain order of gel state bilayers. At a high cholesterol concentration, the gel state is transformed to a liquid ordered phase (Hunter *et al*, 1988). An increase in cholesterol content of the bilayers results in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayers obtained. Presence of charge tends to increase the inter lamellar distance between successive bilayers in multilamellar vesicle structure and leads to greater overall entrapped volume.

Resistance to osmotic stress

Addition of a hypertonic salt solution to a suspension of niosomes brings about reduction in diameter. In hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicles, followed by faster release, which may be due to mechanical loosening of vesicles structure under osmotic stress.

Membrane Composition

The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives. In case of polyhedral niosomes formed from C16G2, the shape of these polyhedral

niosome remains unaffected by adding low amount of solulan C24 (cholesteryl poly-24-oxyethylene ether), which prevents aggregation due to development of stearic hindrance. In contrast spherical Niosomes are formed by C16G2: cholesterol: solulan (49:49:2). The mean size of niosomes is influenced by membrane composition such as polyhedral niosomes formed by C16G2: solulan C24 in ratio (91:9) having bigger size (8.0±0.03mm) than spherical/tubular niosomes formed by C16G2: cholesterol: solulan C24 in ratio (49:49:2) (6.6±0.2mm). Addition of cholesterol molecule to niosomal system provides rigidity to the membrane and reduces the leakage of drug from niosome.

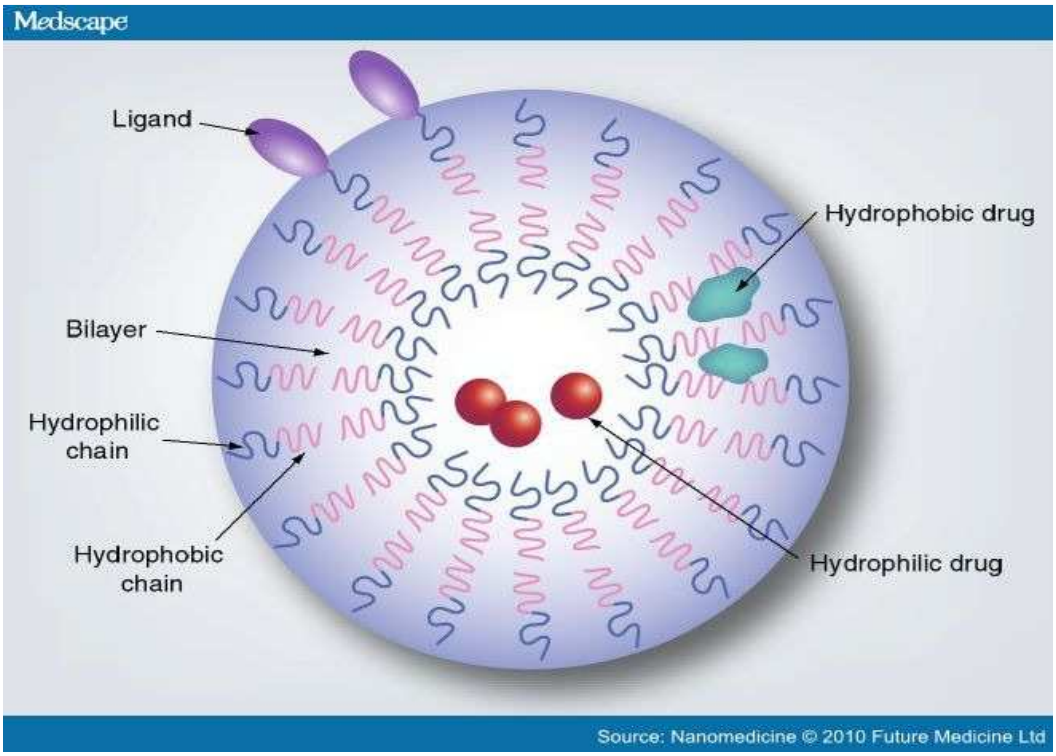


Figure 2a. Structure of niosome

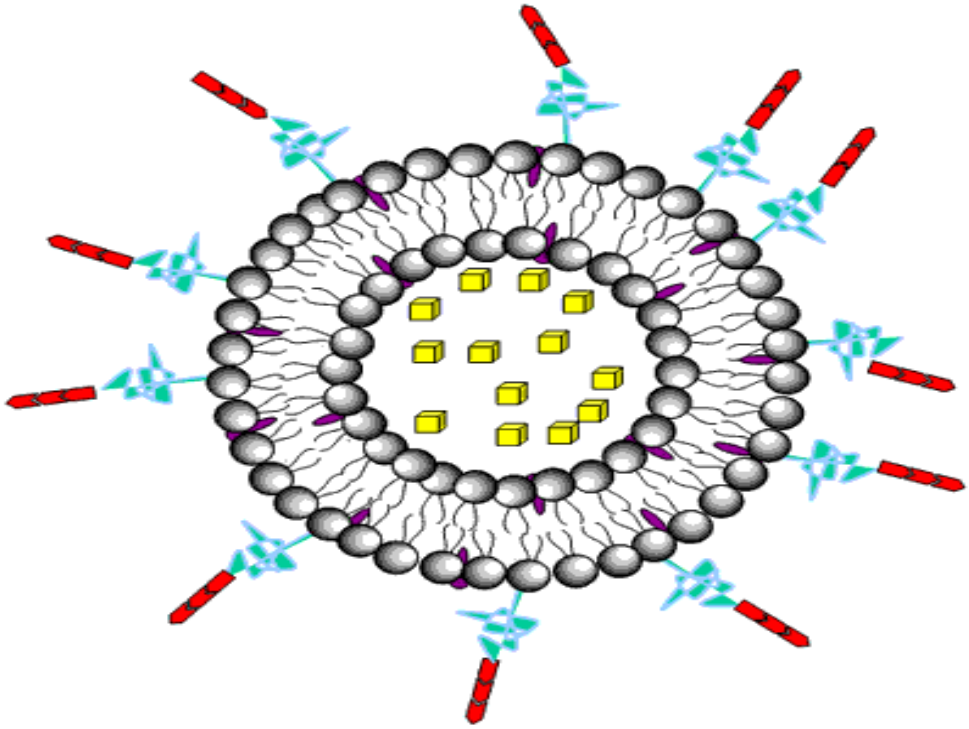


Figure 2b . Struture of niosome

COMPOSITION OF NIOSOMES

In addition to drug the niosome consists of 2 components. The main components are non-ionic surfactants. Membrane additives stabilize the niosomes (Biju *et al*, 2006, Jain *et al*, 2002).

1. Main component : Non-ionic surfactant
2. Membrane additives: Cholesterol, Stabilizer- Charged inducer molecule
3. Drug

3.3 NON- IONIC SURFACTANTS

The non-ionic surfactants are uncharged amphiphilic compounds. The non-ionic surfactants orient in an aqueous medium as planar bilayer lattices wherein polar (or) hydrophilic heads align facing aqueous bulk while hydrocarbon segments are so aligned that their interaction with aqueous media is minimized (Biju *et al*, 2006).

Every bilayer folds over itself to be a continuous membrane that forms vesicles so that hydrocarbon / water interface remains no more exposed.

Examples of non-ionic surfactants forming vesicles are, polyoxy ethylene fatty acid esters, polyoxy ethylene alkyl esters (including ethers of fatty alcohols) polyoxy ethylene sorbitan esters, polyoxy ethylene glyceryl mono and diesters, sucrose diester, propylene glycol stearate, long chain acyl amide, C_{12}-C_{22} fatty alcohols etc.,

BRIJ^{TM} (Polyoxy ethylene fatty acid esters), SPAN^{TM} (Sorbitan fatty acid esters) and TWEEN^{TM} (Polyoxy ethylene derivatives of sorbitan fatty acid esters.) are commercially available amphiphile surfactants.

The choice of non-ionic surfactant on vesicle formation depends on hydrophilic lipophilic balance (HLB), critical micellar concentration (CMC) and critical packing parameter of amphiphiles.

HLB of surfactants

Hydrophilic lipophilic balance (HLB) is a good indicator of the vesicle forming ability of surfactants (Uchegbu ***et al***, 1998).

- With the sorbitan ester (span) surfactants, a HLB number of between 4 and 8 was found to be compatible with vesicles formation.
- Tween 20 having HLB number 16.7 is too hydrophilic to form a bilayer membrane. However with an optimum level of cholesterol it forms niosomes.
- Ether amphiphiles bearing a steroidal C_{14} alkyl (or) C_{16} alkyl groups form vesicles.
- Polyoxy ethylene alkyl ether (Brij) forming vesicles increase six-fold bioavailability for intranasally administered insulin.

- Low phase transition temperature, increased leakage of low molecular weight drugs from the aqueous compartment and decreased stability of the niosomes was observed when hydrophilic surfactants are used.
- High phase transition temperature, decreased leakage of low molecular weight drugs from the aqueous compartment and increased stability of the niosomes was observed with hydrophobic surfactants.

CRITICAL PACKING PARAMETER

The micelle-forming amphiphiles show relatively high solubility in water. The concentration corresponds to CMC. The prediction of vesicle formation characteristics is not just a matter of HLB numbers, CMC values, but involves several other factors Israelachvili *et al*, 1991 suggested that parameters of self assemblages are governed by critical packing parameter (CPP). Their self-organization in water is mainly the result of the hydrophobic effect, as in the case of soap and detergent.It also depends on the relative proportions of hydrophobicity and hydrophilicity of the lipids as well as mesogen molecular geometry. The symmetry of the lipid self-assembly and liquid crystalline-phase formation show strong dependence on the molecular shape of the mesogen/amphiphiles. The different shapes and volumes constructing different phases are characterized by CPP, a dimensionless group (Ucheghu *et al*, 1998, Vyas *et al*, 2004).

The critical packing parameter (CPP) is defined as

$$CPP = \frac{V}{\ell_c . a_o}$$

Where,

V= Hydrophobic group volume

ℓ_c = The critical hydrophobic group length

a_o = The area of hydrophilic head group

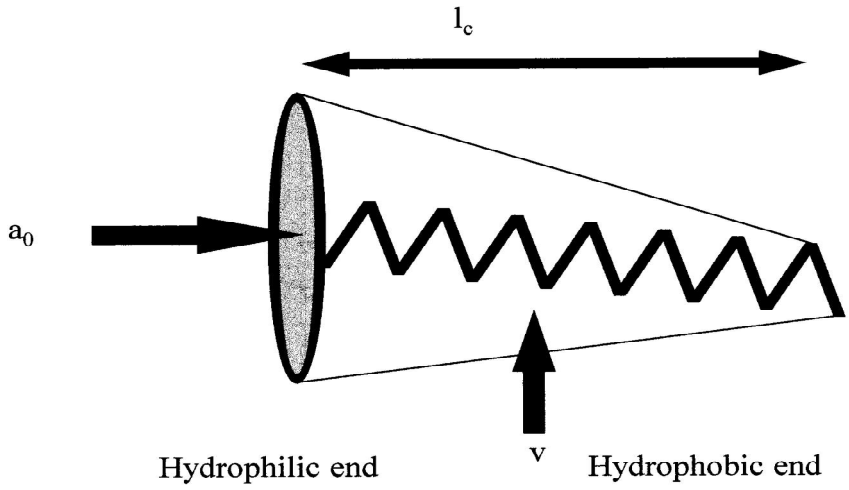


Figure 3. Schematic representation of an amphiphile

The vesicle forming ability of amphiphiles depends on the CPP value which is determined by using hydrophobic group volume and area of hydrophilic head group.

CPP = 0.5-1 -- Surfactant is likely to form vesicles.

CPP < 0.5 -- Large hydrophilic head group give spherical micelles.

CPP > 1.0 -- Large contribution from the hydrophobic group value produces inverted micelles (Biswal *et al*, 2008).

The different structures formed by amphiphilic molecules are represented in figures as follows:

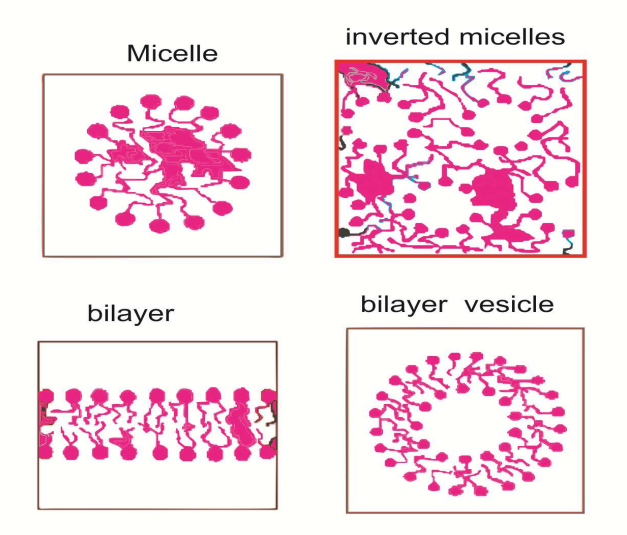


Figure 4. Structures formed by amphiphilic molecules

CHOLESTEROL

Steroids are important components of cell membrane and bring bilayer fluidity and permeability. The most common additive found in niosomal systems is cholesterol which is known to abolish the gel to liquid phase transition of liposomal and niosomal systems, resulting in less leakiness of the vesicles. It may have effects on membrane permeability, encapsulation efficiency, bilayer rigidity, ease of rehydration of freeze dried niosomes and toxicity. A molar ratio of 1:1 between cholesterol and non-ionic surfactants is an optimal ratio for the formation of physically stable niosomal vesicles. Cholesterol can be incorporated in bilayers at significantly higher molar ratio, although by itself it does not form niosomal bilayer. Its -OH group orients towards aqueous phase while the rest of the molecule orients (aliphatic chains) parallel to the hydrocarbon chain of surfactants (Vyas *et al*, 2004, Biswal *et al*, 2008).

Cholesterol is known to have important modulatory effect on the bilayer membrane. Cholesterol acts as fluidity buffer. Below the phase transition it tend to make the membrane less

ordered while above the transition it tends to make the membrane more ordered, thus suppressing the tilts and shift in membrane structure specifically at the phase transition.

Role of cholesterol in bilayer formation

- Acts as a fluidity buffer.
- After intercalation with phospholipid molecules alters the freedom of motion of carbon molecules in the acyl chain.
- Restricts the transformations of trans-to gauche-conformation.

DRUG

The drug is actively or passively entrapped in vesicles. In passive trapping, drug and lipids are co dispersed with fraction of drug being entrapped, according to hydrophobicity and electrostatic charge. If the drug is hydrophilic, it will be entrapped in the internal aqueous phase and the hydrophobic drug will be entrapped in lipid region. Active trapping can be achieved by ion gradients placed across the niosomal membranes. This allows drug entrapment after the niosomal carrier has been formed (Biswal ***et al***, 2008).

The physico-chemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer. The drug interacts with surfactant head groups and develops the charge that creates mutual repulsion between surfactant bilayers and hence increases vesicle size. The aggregation of vesicles is prevented due to the charge development on bilayer.

STABILIZER

One of the methods used to stabilize niosomes is to add a charged inducing molecule to the bilayer. It is used for preventing aggregation of niosomes.

Examples of positive charge inducers

Protamine, Polyamine, Polyvinyl pyridine, Poly oxethane, Poly amidoamines, Cetyl pyridinium chloride, Stearyl amine (STR), Triethanolamine etc.

Examples of negative charge inducers

Oleic acid, Palmitic acid, Dicetyl phosphate (DCP), Cetyl sulphate, Phosphatidic acid, Phosphatidyl serine etc,

The charged molecule is added in niosomal formulation in an amount of 2.5–5 mol%. A higher concentration of charged molecules may inhibit the formation of niosomes. It is added in stable niosomal dispersion which exhibits a constant particle size and a constant level of entrapped drug. There must be no precipitation of the membrane components this treatment (Gopinath *et al*, 2004, Madhurilatha *et al*, 2011).

The inclusion of a charged molecule in the bilayer shifts the electrophoretic mobility making it positive or negative and prevents niosomes aggregation.

3.4 TYPES OF NIOSOMES

They are divided in to three types as follows,

1. Multilamellar niosomes (>0.05μm)
2. Small unilamellar niosomes (0.025-0.05μm)
3. Large unilamellar niosomes (>0.01μm)

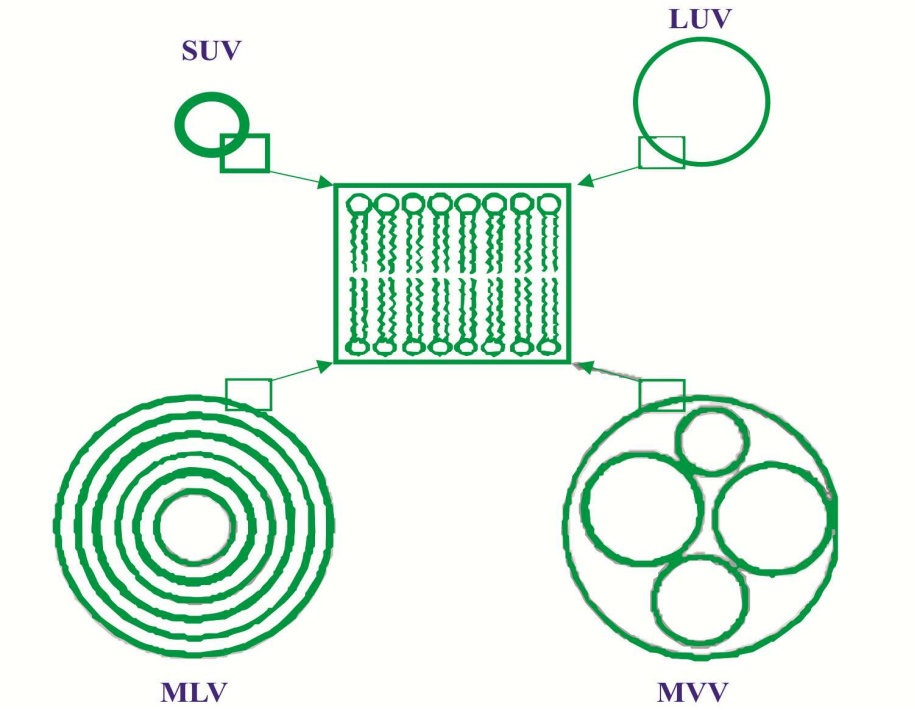


Figure 5. Schematic illustration of different size and number of lamellae

SUV: Small unilamellar vesicles **LUV:** Large unilamellar vesicles

MLV: Multilamellar vesicles **MVV:** Multi vesicular systems

3.5 METHODS OF PREPARATION OF NIOSOMES

1. Ether Injection Method
2. Hand Shaking Method (Thin film hydration technique)
3. Sonication
4. Microfluidization
5. *Multiple Membrane Extrusion method*
6. *Reverse Phase Evaporation Technique (REV)*
7. Trans membrane pH gradient Drug Uptake Process
8. The “Bubble” method
9. Ethanol injection method

10. Formation of niosomes from proniosomes
11. Emulsion method
12. Lipid injection method
13. Niosome preparation using micelle

1. Ether Injection Method

This method provides a means of making niosomes by slowly injecting the surfactant/cholesterol mixture (dissolved in diethyl ether) in to the aqueous phase maintained at 60^0 through 14-gauge needle. This method produces unilamellar vesicle with highest entrapment efficiency. Depending upon the conditions used, the diameter of the vesicles range from 50-1000nm (Rakesh *et al*, 2007, Alamayehu *et al*, 2010).

2. Hand Shaking Method (Thin film hydration technique)

The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flak. The solvent is evaporated at constant low temperature using a rotary evaporator, leaving behind a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film is rehydrated with aqueous phase maintained at 0-60 ºC with gentle agitation (Vyas *et al*, 2004, Rakesh *et al*, 2007, Pranshu *et al*, 2011).

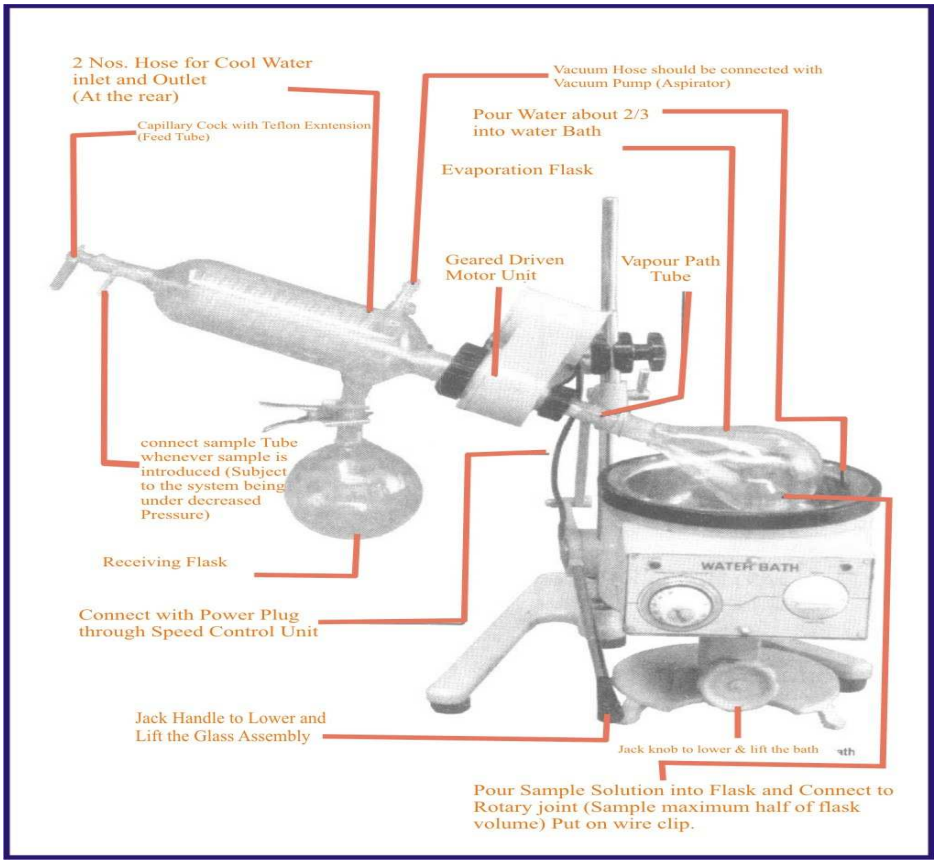


Figure 6. Thin film hydration method

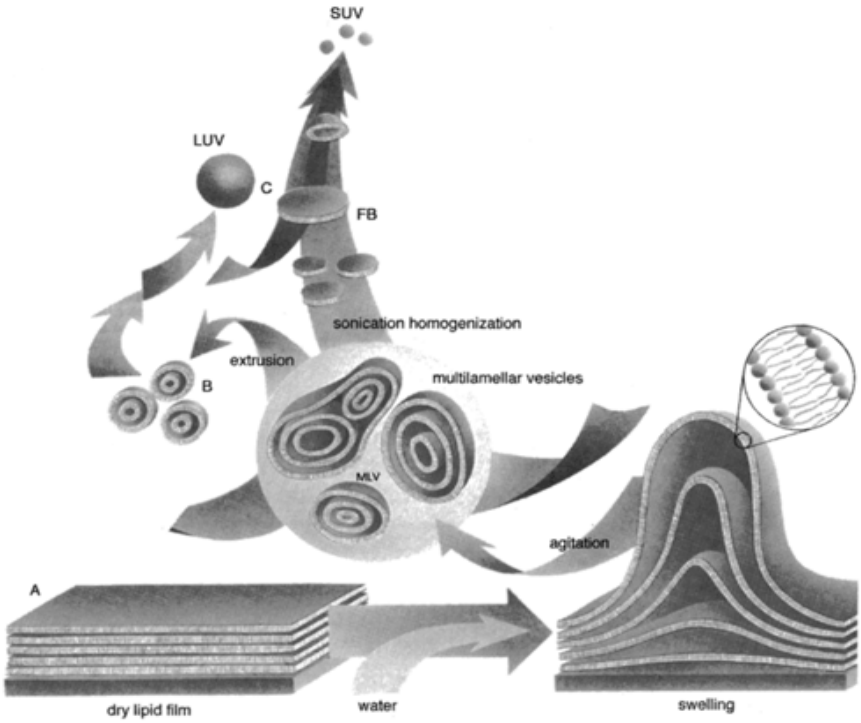


Figure 7. Mechanism of formation of niosomes

The lipids are cast as stacks of film from their organic solution in rotary flash evaporator under reduced pressure. The cast film is dispersed in an aqueous medium by hydration of the lipids (surfactants and cholesterol) which swell, peel off from the wall of the round bottom flask, become vesiculate and form multilamellar vesicles. The energy required for the swelling of the lipids and dispersion of lipid film is imparted by agitation.

Thermo sensitive niosomes are prepared by evaporating organic solvent there by leaving behind a thin film of lipid on the wall of rotary flask. The aqueous phase, containing drug is added slowly by shaking at room temperature followed by sonication.

3. Sonication

The surfactant/cholesterol mixture in organic solvent is mixed with aqueous phase in a vial. Then the mixture is sonicated at 60^0 for 3 minutes to produce niosomes. The vesicles produced are unilamellar and smallest in size (Sankar *et al*, 2009, 2010, Subodh *et al*, 2010).

4. Micro fludization

Microfludization is used for preparing unilamellar vesicles of a defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities (up to 1700 ft/sec) in a precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed (Pranshu *et al*, 2011, Dubey *et al*, 2010, Rakesh *et al*, 2007).

5. **Multiple Membrane Extrusion method**

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug solution and the resultant suspension is extruded through polycarbonate membranes which are placed in series up to 8 passages. It produces niosomes of uniform size (Rakesh *et al*, 2007, Madhurilatha *et al*, 2011).

6. Reverse Phase Evaporation Technique (REV)

The novelty in this method is the removal of solvent from an emulsion by evaporation. Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phase system is sonicated at 4-5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 minutes to yield niosomes (Pranshu *et al*, 2011, Navin *et al*, 2011).

7. Trans membrane pH gradient Drug Uptake Process

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 mM citric acid (pH 4.0) by vortex mixing. The resulting multilamellar vesicles are then treated to three freeze thaw cycles and sonicated. To this niosomal suspension, aqueous solutions containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with the addition of 1M disodium phosphate (this causes the drug which is outside the vesicle to become non-ionic and can then cross the niosomal membrane, and once inside it is again ionized thus not allowing it to exit the vesicle). This mixture is later heated at 60°C for 10 minutes to give niosomes (Rakesh *et al*, 2007, Dubey *et al* 2010).

8. The "Bubble" Method

It is novel technique for the preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion is mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas (Rakesh *et al*, 2007, Dubey *et al*, 2010).

9. Ethanol injection method

This method has been reported for the preparation of small unilamellar vesicles (SUVs) without sonication. In this method, an ethanol solution of surfactant is injected rapidly through a fine needle into excess of saline or other aqueous medium. Vaporization of ethanol leads to the formation of vesicles (Navin *et al*, 2011).

10. Formation of niosomes from proniosomes

A water-soluble carrier (e.g. sorbitol) is coated with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation termed as "proniosomes". The niosomes are reconstituted by the addition of aqueous phase with a T > Tm transition with brief agitation (Sudhamani *et al*, 2010, Almira *et al*, 2001).

T – Temperature

Tm – Mean phase transition temperature

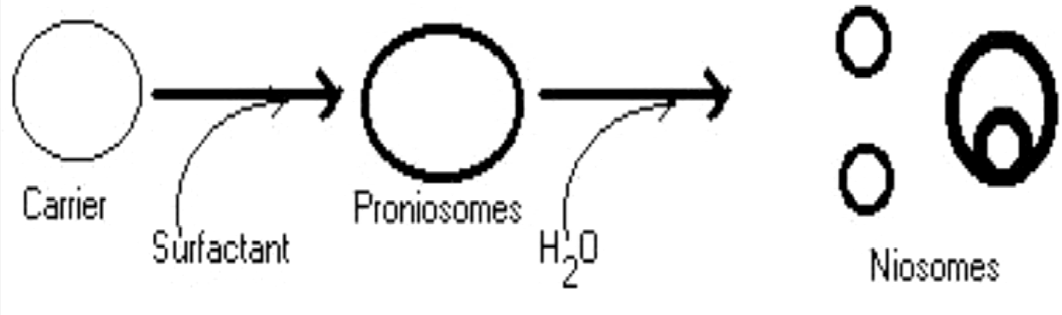


Figure.8 Formation of niosomes from proniosomes

The formulation of niosomes from maltodextrin based proniosomes was reported Walsh et al, 1989.This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water (Chengjiu *et al*, 1999).

11. Emulsion method

The oil in water emulsion is prepared from an organic solution of surfactant, cholesterol and an aqueous solution of the drug. The organic solvent is then evaporated, leaving behind niosomes dispersed in the aqueous phase (Kazi *et al*, 2010).

12. Lipid injection method

This method does not require expensive organic phase. Here, the mixture of lipids and surfactant is first melted and then injected into a highly agitated, heated aqueous phase containing dissolved drug. The drug can be dissolved in molten lipid and the mixture injected into agitated, heated aqueous phase containing surfactant (Kazi *et al*, 2010).

13. Niosome preparation using micelle

Niosomes may also be formed from a mixed micellar solution by the use of enzymes. A mixed micellar solution of C16G2, dicalcium hydrogen phosphate, polyoxyethylene cholesteryl sebacetate diester (PCSD) converts to a niosome dispersion when incubated with esterases. PCSD is cleared by the esterases to yield polyoxyethylene, sebacic acid and cholesterol. Cholesterol in combination with C16G2 and DCP then yields C16G2 niosomes (Amritha *et al*, 2006)

3.6 THE REDUCTION OF NIOSOME SIZE

A reduction in vesicle size may be achieved by

- Probe sonication
- Extrusion through 100nm nucleopore filter
- The combination of sonication and filtration
- Micro fluidizer
- High pressure homogenizer.

3.7 SEPERATION OF UNENTRAPPED DRUG

In vesicular system half of the drug is encapsulated and half is external to the niosomes and may eventually yield systems with a beneficial biphasic bidistribution profile (Uchegbu *et al*,

1998, Rakesh *et al*, 2007, Kumar *et al*, 2011). The removal of unentrapped solute from the vesicles can be accomplished by

1. Dialysis

The aqueous niosomal dispersion is dialyzed in dialysis tubing against phosphate buffer or normal saline or glucose solution.

2. Gel Filtration

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and elution with phosphate buffered saline or normal saline.

3. Centrifugation

The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.

3.8 CHARACTERIZATION OF NIOSOMES

1. Entrapment efficiency

The unentrapped drug is separated by dialysis, centrifugation or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug. Where,

$$\% \text{ drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant})}{\text{Total drug}} \times 100$$

2. Vesicle diameter

Niosomes assume spherical shape and so their diameter can be determined using light microscopy, photon correlation microscopy, and scanning electron microscopy and freeze fracture electron microscopy (Biju *et al*, 2006, Navin *et al*, 2011)

3. *In-vitro* release

A method of *invitro* release study includes the use of dialysis tubing. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is pipetted into a bag made up of the tubing and sealed (Kasi *et al*, 2010). The bag containing the vesicles is placed in buffer solution in a beaker with constant shaking at 25°C or 37°C. At various time intervals, the buffer is analyzed for the drug content.

3.9 ROUTES OF ADMINISTRATION

Azmin *et al*, 1985, investigated niosomal delivery of methotrexate to mice by oral and intravenous administration. On oral administration absorption of methotrexate was significantly increased.

Hofland *et al*, 1992, studied transdermal delivery of estradiol entrapped within niosomes. Brewer and Alexander performed studies on adjuvant activity of niosomes on the BALB/C humoral response to bovine serum albumin after intraperitoneal and subcutaneous administration.

***3.9 In vivo* BEHAVIOUR OF NIOSOMES**

in vivo niosomes have been found to be equiactive to liposomes in improving the therapeutic performance of drug and their distribution in the body. Like liposomes, niosomes are taken up by the liver and break down to release the free drug which re enters the circulation and maintains the plasma drug level.

Parthasarathi *et al*, 1994, found niosomes to be stable in plasma. Moser *et al* found niosomes bearing hemoglobin to be physically stable and albumin, transferrin were absorbed on vesicles without destabilizing them.

3.11 CHARACTERIZATION OF NIOSOMES

Table I. Analytical methods for characterizing niosomes

S.NO.	PARAMETER(S)	METHOD(S)
1.	Morphology	Transmisson Electron Microscopy, Scanning Electron Microscopy, Optical Microscopy (OM), Cryo- Scanning Electron Microscopy, Freeze Fracture Microscopy
2.	Vesicle size determination and Size distribution	Dynamic Light Scattering using particle Size Analyzer(PSA), Malvern Master Sizer, Photon Correlation Spectroscopy (PCS), OM, SEM, Laser Diffraction PSA
3.	Zeta potential/ Surface Charge	Micro-electrophoresis meter, High Performance Capillary electrophoresis and Malvern Zeta Sizer (Zetameter)
4.	Rheological Properties (Elasticity)	Ostwald's U-tube, Low shear Rheo Analyzer and Extrusion method
5.	Viscosity	Ostwald's viscometer
6.	Membrane micro-structure	Negative Staining TEM
7.	Lamellarity	OM, TEM
8.	Bilayer spacing and thickness	X-Ray Scattering Analysis
9.	Gel-Liquid transition temperature & Thermal Analysis	Differential Scanning calorimetry, Differential Thermal Analysis and Hot Stage Microscopy
10.	Circular Dichroism	Spectropolarimeter
11.	Micropolarity measurement	Fluorescence Spectrophotometer
12.	Fluidity of vesicles	Differential Polarized Phase Fluorimetry
13.	Turbidity measurement	UV-Visible Diode Array Spectrophotometer
14.	Entrapment Efficiency	Centrifugation method, Dialysis method, Gel Exclusion Chromatography
15.	In-vitro release rate	Using dialysis membrane
16.	Permeation study	Franz Diffusion Cell
17.	Conductivity	Conductometer

3.12 TOXICITY AND STABILITY

Non-ionic surfactants used in niosomes are non-toxic and no toxic effects have been reported in animal studies due to the use of niosomes as drug carriers.

Jain *et al*, 1995, reported that there are no morphological changes on storage for three months. Baille *et al*, 1986, determined the stability in buffer and reported that the entrapped solute would be retained under long term storage conditions.

3.13 STUDIES ON NIOSOMES AND THEIR MEDICINAL APPLICATIONS

Table II

S. NO.	APPLICATIONS	DRUGS STUDIED
1.	Cancer chemotherapy and targeted drug delivery	Doxorubicin, Danorubicin Hcl, Methotrexate (MTX), 5-flurouracil, Adiramycin, Vincristine, Cytarabine Hcl
2.	Transdermal drug delivery	Nimesulide, Lidocaine, Cyclosporine, Estradiol, Erythromycin, α-interferon, Indomethacin, Enoxacin, Finasteride
3.	Enhancement of bioavailability	Diclofenac, Flurbiprofen, Bleomycin, Vincristine, Doxorubicin, Acetazolamide
4.	Ocular drug delivery	Timolol maleate, Acetazolamide, Cyclopetolate
5.	Pulmonary drug delivery	All trans retinoic acid (ATRA)
6.	Brain Targeted Drug Delivery	VIP loaded glucose bearing niosomes
7.	Protein/Peptide and Hormone delivery	LHRH, Insulin (oral), 9-desglycinamide -8-arginine vasopressin (DGAVP)
8.	Local/Intra Articular drug delivery	Radiolabelled Diclofenac Na niosomal vesicles
9.	Enhancement of stability improved photo stability	DGAVP, Haemoglobin, Dithranol, β-carotene
10.	Improved thermal and oxidative stability	β-carotene
11.	Prolonged release	Propranolol Hcl, Doxorubicin
12.	For improved anti-infective therapy	Sodium stibogluconate, Rifampicin
13.	Immuno stimulatory niosomes (antigenic)	Haemagglutinin, Ovalbumin, Hepatitis B DNA vaccine niosomes, Plasmid DNA encoding proteins of Hepatitis B virus, Influenza DNA vaccine niosomes and Tetanus Toxoid Niosomes
14.	Diagnosis	Urokinase
15.	Radio-pharmaceutical carrier and imaging study.	Iobitridol (X-ray imaging studies), and Iopromide (Kidney imaging studies)

3.14 OTHER APPLICATIONS

(a) Sustained release

Niosomes can provide relatively constant and sustained plasma level of drug. Sustained action of niosomes could be applied to drugs with low therapeutic index.

(b) Localized drug action

Localization of drug action results in enhancement of efficacy or potency of the drug and at the same time reduces its systemic toxic effects. Niosomes are a promising vehicle for drug delivery and being non-ionic it is less toxic and improves the therapeutic index of drug by restricting its action to target cells (Alamayehu *et al*, 2010, Madhav *et al*, 2011).

3.15 ENHANCEMENT OF BIOAVAILABILITY

The lipophilic form of drug has enhanced membrane/water partition coefficient as compared to the hydrophilic form of the drug. A big advantage of increased bioavailability through increased lipophilicity is reduction in drug dose.

CHAPTER-IV

TRANSDERMAL DRUG DELIVERY SYSTEM

INTRODUCTION

Transdermal drug delivery system is one among of the various modes of drug delivery which facilitates passage of therapeutic quantities of drug substances through the skin and for systemic (or) local effects (Loyd.V *et al.,* 2005).

Discovering a new medicine is a very expensive and time-consuming work. But however, redesigning the modules and means to transport medicine into the body is a less demanding and more lucrative task.

In the normal drug release, if the medication may not be absorbed means it will release too slowly (or) if it delivered too fastly means, the patient may suffer untoward effects. To rectify the above drawback one of the solutions developed was transdermal drug delivery systems.

Human skin is a uniquely engineered organ that permits terrestrial life by regulating heat and water loss from the body whilst preventing the ingress of noxious chemicals or microorganisms. It is also the largest organ of the human body, providing around 10% of the mass of an average person, and it covers an average area of $1.7m^2$. Whilst such a large and easily accessible organ apparently offers ideal and systemic actions, human skin is a highly efficient self-repairing barrier designed to keep the insides in and the outside out.

Skin membranes can be examined at various levels of complexity, the membranes can be regarded as a simple physical barrier more complexity can be introduced by viewing skin as

various barriers in series. We can then introduce barriers in parallel by considering drug transport through pores in the tissue.

4.1 SKIN

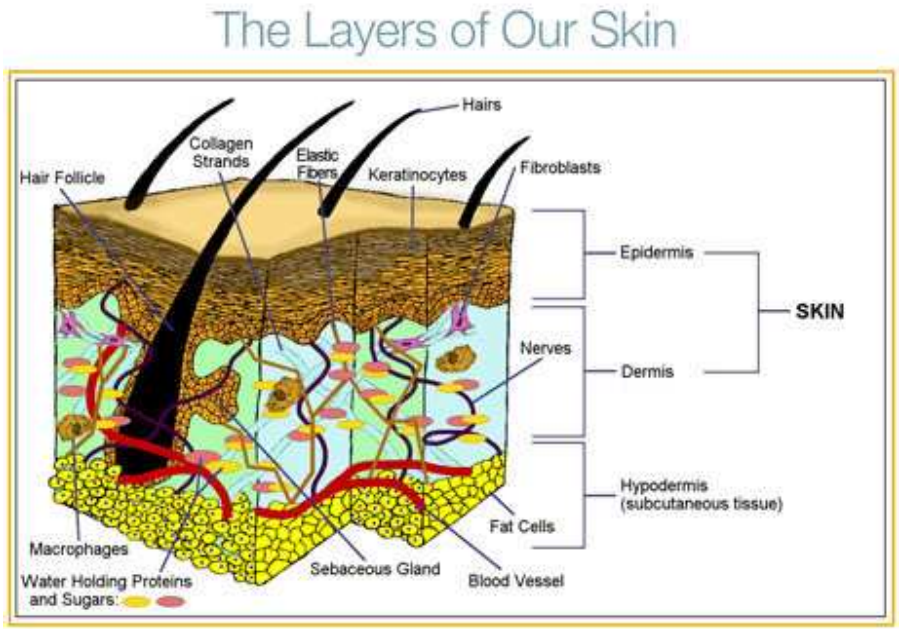


Figure 9. Structure of skin

Skin is the largest organ of 1.5 to 2 m^2 in adult which covers the whole body. Thickness of skin varies from place to place i.e. it is so thick in palm, foot and so thin in eyelid.

The skin is broadly classified into two layers. They are; A) epidermis and B) Dermis.

A. Epidermis

It is the most superficial (or) outermost layer of skin. The cells in the epidermis shed periodically and replaced by new cells usually a complete replacement of epidermis takes about 40 days (Vyas.S.P *et al,* 2005).

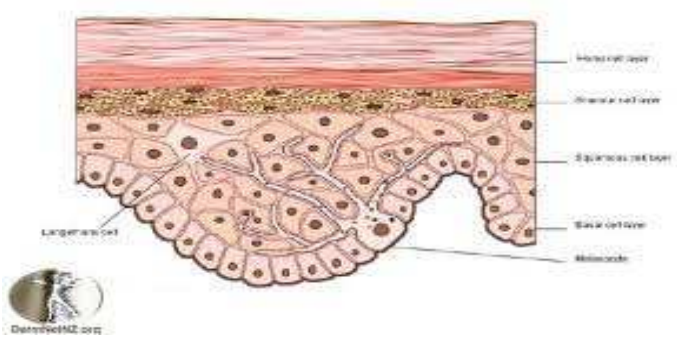


Figure 10. structure of epidermis

Various Layers In Epidermis

There are about four layers. They are;

i. Stratum corneum.

ii. Stratum lucidum.

iii. Stratum granulosm.

iv. Germinative layer.

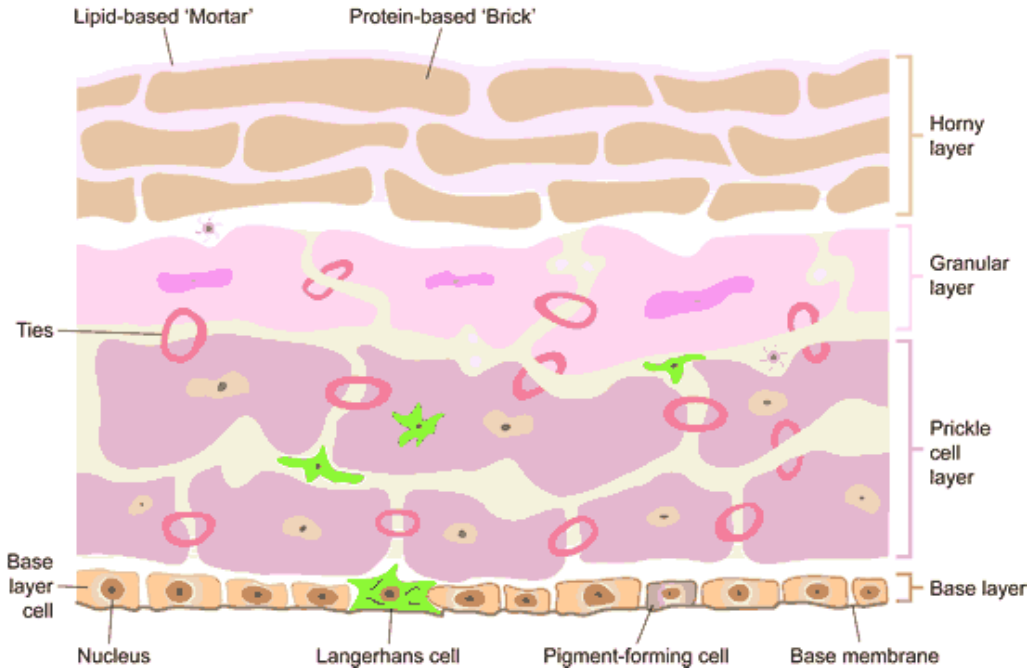


Figure 11. Structure of stratum corneum

B. Dermis

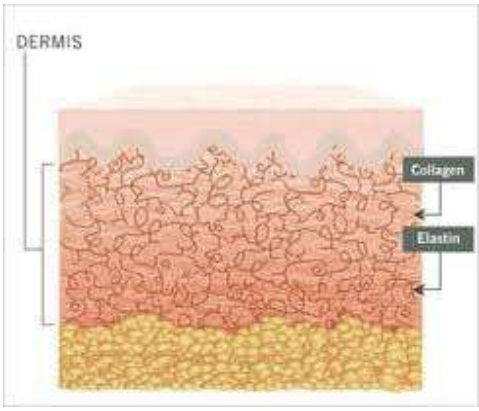


Figure 12. Structure of stratum corneum

Dermis consists of the following things in it

- Blood vessels.
- Lymph vessels.
- Sensory (somatic) nerve ending.
- Sweat glands and their ducts.
- Hair roots, hair follicles and hairs.
- The arrectores pilorum – involuntary muscles attached to the hair follicles.
- Sebaceous glands.

Heirs, secretions from sebaceous glands and ducts of sweat glands pass via the epidermis to reach the surface.

4.2 FUNCTION OF SKIN

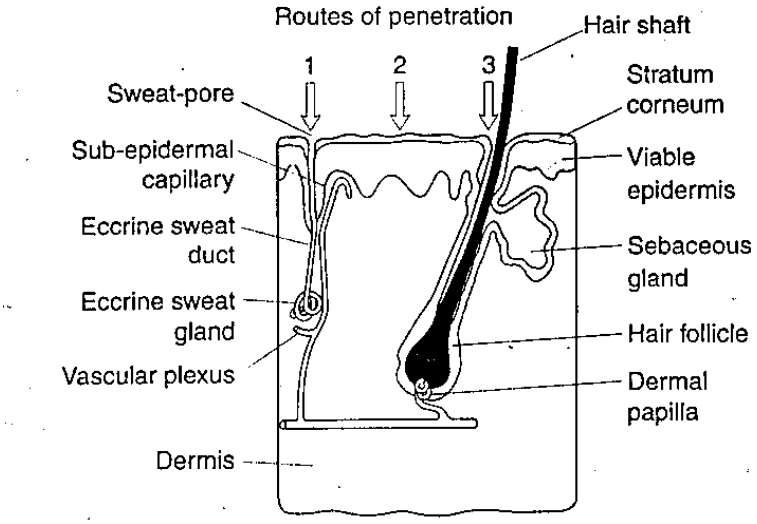
It does major functions to the human body. They are

- Mechanical function
- Protective function
-

- microbiological barrier
- Chemical barrier
- Radiation barrier
- Electrical Barrier

- Regulation of body temperature
- Formation of vitamin D
- Sensation
- Absorption
- Excretion

4.3 RATIONAL APPROACH TO DELIVER THE DRUG VIA SKIN Fig.no:11



There are three main ways to approach the problem of formulating a successful topical dosage form.

1. Manipulating the barrier function of the skin
2. Directing drugs to the viable skin tissues without using oral, systemic (or) other routes.
3. Using skin delivery for systemic treatment.

Dermatologist aim five main target regions. They are skin surface, horny layer, viable epidermis & upper dermis, skin glands and systemic circulation.

4.4 FACTORS AFFECTING DRUG DELIVERY

Transdermal route should have the capability to deliver the drug, regardless of size (or) structure at a predetermined rate. But there are some factors which influence the rate of drug delivery (There are two types of factors

They are as follows

A. Biological factors

- Skin condition.
- Skin age.
- Amount of blood flow.
- Regional Skin sites.
- Skin metabolism.
- Species differences.

B. Physiochemical Factors

- Skin hydration.
- Temperature and pH
- Diffusion Coefficient
- Drug applying surface area.
- Drug Concentration.
- Partition Coefficient.
- Molecular size and shape.

4.5 TYPES OF TREATMENT ACHIEVED BY TOPICAL DRUG DELIVERY

- Camouflage.
- Protection effects,.
- Insect repellant.
- Antimicrobial.
- Antifungal.
- Emolliency.
- Keratosis.
- Antiperspirant.
- Exfolient.
- Antibiotic.
- Depilatory.
- Anti inflammation.
- Anti pruritic.
- Local anesthetic.
- PUFA and PDT.
- Anti histamine.
- Anti angina.
- Anti-ischaemic.

4.6 VARIOUS TYPE OF DOSAGE FROM USED IN TOPICAL DRUG DELIVERY

- Liquid preparations.
- Gels (jellies).
- Powders.

- Ointments.
- Creams.
- Paste.
- Aerosols.
- Poultice.
- Transdermal patch.

4.7 METHODS TO INCREASE PERCUTANEOUS ABSORPTION

Chemical penetration enhancers such as

- Solvents
- Alkyl Methyl sulphoxides
- Pyrrolidones
- Azone and related compounds
- Surfactants
- Ionophoresis
- Sonophoresis
- Electrophoresis
- Laser Ablation
- Prodrugs

4.8 ADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEM

1. Avoidance of significant presystemic metabolism (degradation in GI tract (or) Liver, gut wall) and the daily drug dose is reduced(Vyas.S.P *et al.,*2002).
2. Reduction of Inter and Intra Patient variability.
3. Drug level can be maintained in systemic circulation within the therapeutic window.

4. Drug action is extended and frequency of administration is reduced.
5. Improved patient compliance.
6. Drug input can be easily terminated.
7. Increased safety.
8. Greater convenience.
9. Drugs with short biological half lives.

4.9 DISADVANTAGES OF TRANSDERMAL DRUG DELIVERY SYSTEM

1. Skin irritation.
2. Skin allergy.
3. Potent drugs cannot be given by this route.

4.10 ROLE OF NIOSOMES IN TRANSDERMAL DRUG DELIVERY SYSTEM

Niosomes can be used to deliver both hydrophobic and hydrophilic drugs via transdermal route. Although niosomes were tried for various routes it is used in the market for transdermal route (Novasome Products Such as 30% Petrolatum Novasomes and 10% Salicyclic Acid Novasomes). Studies showed that an enhanced delivery of drugs when encapsulated in niosomes. Niosomes increase skin penetration of drugs and it can act as local depot for sustained release of dermally active compounds. When non ionic surfactants are incorporated into niosomes they are much better tolerated by the skin then when they are used in emulsion (Jia – Y – Fang *et al,* 2001).

4.11 VARIOUS BIO ACTIVE AGENTS WHICH ARE TRIED VIA TRANS DERMAL ROUTE AS NIOSOME DRUG DELIVERY SYSTEM

- Cyclosporin – A.
- Lidocaine.

- Estradiol.
- Erythromycin.
- Alpha – interferon.
- Diclofenac sodium.
- Nimesulide.
- Enoxacin.
- Miconazole nitrate.
- Ketoconazole.
- Tretionin.
- Metronidazole.

CHAPTER-V

LITERATURE REVIEW

Raju Jukanti *et.al.,* 2011, developed a oral delivery of valsartan from maltodextrin based proniosome powders .The proniosome powders were prepared by varying the ratio of span 60 and cholesterol and evaluated for micromeritic properties and the results indicate acceptable flow properties. The formulation containing equimolar ratio of span 60 and cholesterol showed smaller vesicle size, high surface charge and entrapment efficiency. The formation of noisomes and surface morphology of optimized proniosome formulation was studied by optical and scanning electron microscopy, respectively. FT-IR, differential scanning calorimetry, and powder X-ray diffraction studies performed to understand the solid state properties of the drug reveal the absence of chemical interaction, drug transformation from crystalline to amorphous and molecular state. The invitro dissolution study carried out in both simulated gastric and intestinal fluid demonstrates improved dissolution characteristics compared to pure drug. The augment in permeation enhancement from proniosome formulation across rat intestine suggest the potential of proniosome carriers for improved oral delivery of valsartan.

Phikunthong Kopermsub *et al.,* 2011, prepared a niosome for encapsulation of nisin and EDTA and their antibacterial activity enhancement. Different formulations of niosomes were prepared using span 80 with sodium stearoyl lactate (SSL) and PEG400 as additives .The corresponding niosomes were used for encapsulation of antibacterial agents including nisin and EDTA .Characterization of unloaded and loaded niosomes was carried out by means of size, zeta potential, and also encapsulation efficiency and antibacterial activity specifically for loaded niosomes. Unloaded niosomes were stable at least 2 months when temperature challenge was

applied at 4^0 C, RT, and 45^0 C. An addition of 0.5mM SSL, both with and without PEG was found to enhance stability of loaded niosomes throughout 2-months of storage. Effect of niosome formulation to zeta potential and encapsulation efficiency of nisin and EDTA was discussed in this paper. A 96- well plate assay was used to assess the antibacterial activity between free and encapsulated forms of nisin and EDTA against *Staphylococcus aureus* and *Escherichia coli.*

Ammar.O.H *et al.,* 2011, formulated a proniosomes as a carrier system for transdermal delivery of tenoxicam. Proniosomes offer a versatile vesicle delivery concept with the potential for drug delivery via the transdermal route. In this study, different proniosomal gel bases were prepared, characterized by light microscopy, revealing vesicular structures, and assessed for their drug entrapment efficiency, stability, and their effect on invitro drug release and ex vivo drug permeation. The lecithin-free Proniosomes prepared from Tween 20: Cholesterol (9:1) proved to be stable with high entrapment and release efficiencies. The in vivo behavior of this formula was studied on male rats and compared to that of the oral market product. The investigated tenoxicam loaded proniosomal formula proved to be non- irritant, with significantly higher anti-inflammatory and analgesic effects compared to that of the oral market tenoxicam tablets.

Mansoor A. Khan *et al.,* 2011, developed a process understanding of a novel pediatric anti-HIV tenofovir niosomes with a high-pressure homogenizer. A variety of factors were systemically evaluated in order to establish the characteristics of the niosomes obtained with a high-pressure homogenizer. The vesicular sizing parameters, electrical properties, drug entrapment data and drug release characteristics were investigated using two groups of factors. The first group presented the physical process variables such as pressure of the homogenizer and the times that the samples were processed (cycles). The second group encompassed the compositional variables such as the drug loading, surfactant chain length, cholesterol level and the level of the charge

imparting agent. The obtained data showed that the drug distributed within both the aqueous and lipid phases of the formed niosomes. Saturation-like behaviors for both the effect of homogenization cycles on the produced size and the effect of the pressure on the size homogeneity were recorded. In contrast to the drug entrapment and conductivity of the niosomal suspension, the vesicular size parameters as well as the zeta potential were inversely proportional with the homogenization parameters. Drug release was significantly affected by the compositional factors rather than the physical ones. The current study demonstrated the usefulness of the microfluidization for the production and further scale-up of anti-HIV niosomes with very small mean vesicular sizes.

Raju Jukanti *et al.,* 2011, studied the enhanced bioavailability of exemestane via proliposomes based transdermal delivery. The prepared proliposomes were characterized for size, zeta potential, and entrapment efficiency. The size of the vesicles was found to be between 440 and 700nm with high entrapment efficiency for the formulation containing greater amounts of phosphatidylcholine. DSC and FTIR studies were performed to understand the phase transition behavior and mechanism for skin permeation, respectively. The drug release across cellophane membrane follows zero-order kinetics by diffusion.Ex vivo permeation enhancement assessed from flux, permeability coefficient, and enhancement ratio were significantly higher for proliposomes gels compared with control. A significant improvement in the bioavailability (2.4-fold). The stability data reveal that the formulations are more stable when stored at 4^0 C.

Tetsuo Minamino *et al.,* 2011, development of anti-EGF immuno liposomes for the treatment of breast cancer. In the present study, we developed novel immuno liposomes targeting HB-EGF for cancer therapy. The immuno liposomes significantly associated with Vero-H cells over expressing HB-EGF compared with their binding to wild-type Vero cells, whereas liposomes

without modification by the antibody did not associate with either type of cells. Moreover, enhanced uptake of the immuno liposomes into Vero-H cells was observed as well as that into MDA-MB-231 human breast cancer cells, which are known to highly express HB-EGF. These results suggest that HB-EGF mediates the binding and uptake of the immune liposomes in HB-EGF-expressing cells. Next, we determined the therapeutic effect of these immune liposomes encapsulating an anticancer drug on tumor-bearing mice. For this purpose, we prepared doxorubicin (DOX)-encapsulated immune liposomes and injected them intravenously into mice bearing MDA-MB-231 cancer cells. As a result, these DOX-encapsulated immune liposomes suppressed not only tumor progression but also tumor regression. In conclusion, our results indicate that anti-HB-EGF antibody-modified liposomes could be a useful DDS carrier for the treatment of HB-EGF expressing cancer.

Nahum Allon *et al.*, 2011, developed a new liposome-based gene delivery system targeting lung epithelial cells using endothelin antagonist. We formulated a new gene delivery system based on targeted liposomes. The efficacy of the delivery system was demonstrated in invitro and in vivo models. The targeting moiety consists of a high-affinity 7-aminoacid peptide, covalently and evenly conjugated to the liposome surface. The targeting peptide acts as an endothelin antagonist, and accelerates liposome binding and internalization. It is devoid of other biological activity. Liposomes with high phosphatidyl serine (PS) were specially formulated to help their fusion with the endosomal membrane at low pH and enable release of the liposome payload into the cytoplasm. A DNA payload, pre-compressed by protamine, was encapsulated into the liposomes, which directed the plasmid into the cell's nucleus. Upon exposure to epithelial cells, binding of the liposomes occurred within 5–10 min, followed by facilitated internalization of the complex. Endosomal escape was complete within 30 min, followed by DNA accumulation in the

nucleus 2 h post-transfection. A549 lung epithelial cells transfected with plasmid encoding for GFP encapsulated in targeted liposomes expressed significantly more protein than those transfected with plasmid complexed with Lipofectamine. The intra-tracheal instillation of plasmid encoding for GFP encapsulated in targeted liposomes into rat lungs resulted in the expression of GFP in bronchioles and alveoli within 5 days.

Varaporn Buraphacheep Junyaprasert *et al.*, 2011, evaluated the physiochemical properties and skin permeation of Span 60 / Tween 60 niosomes of ellagic acid. The aim of the present study was to develop niosomal formulations obtained from the mixture of Span 60 and Tween 60 that could encapsulate EA for dermal delivery. The EA-loaded niosomes were prepared with 1:0, 2:1, 1:1, 0:1 Span 60 and Tween 60, using polyethylene glycol 400 (PEG 400), propylene glycol (PG) or methanol (MeOH) as a solubilizer. The influence of formulations on vesicle size, entrapment efficiency and stability of EA-loaded niosomes was investigated. It was found that all ratios of surfactants could produce EA-loaded niosomes when using 15% (v/v) PG, 15% (v/v) PEG 400 or 20% (v/v) MeOH. The niosomes were spherical multilamellar vesicles showing the localization of EA in the vesicles. The vesicle sizes of the niosomes after extrusion were 124–752 nm with PI less than 0.4. The percentages of entrapment efficiency (% E.E.) of all EA-loaded niosomes varied between 1.35% and 26.75% while PEG 400 niosomes gave the highest % E.E. The most stable and highest entrapped formulation was 2:1 Span 60 and Tween 60 niosomes. Additionally, the in vitro skin permeation revealed that the penetration of EA from the niosomes depended on vesicle size, the amount of EA entrapped and the added solubilizer which could act as a permeation enhancer. From skin distribution study, the EA-loaded niosomes showed more efficiency in the delivery of EA through human epidermis and dermis than EA

solution. The results indicated that the Span 60 and Tween 60 niosomes may be a potential carrier for dermal delivery of EA.

Abdul Hasan Sathali *et al.,* 2011, formulation and evaluation of in situ gelling system of levofloxacin. Levofloxacin is an antibacterial agent which exhibits rapid precorneal elimination and poor ocular bioavailability, when given in the form of conventional ophthalmic solutions. To overcome this, an attempt has been made to formulate pH-triggered in situ gelling system of levofloxacin to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in pH. The levofloxain in situ gelling system formulated by using poly acrylic acid (Carbopol 940) in combination with hydroxyl propyl methyl cellulose (HPMC) which acted as viscosity enhancing agent. The developed formulation was stable, non-irritant and provided sustained release over 8-hour period and it is a viable alternative to conventional eye drops.

Ahmed S. Zidan *et al.,* 2011, studied the characteristics of the niosomes obtained with a high-pressure homogenizer. The vesicular sizing parameters, electrical properties, drug entrapment data and drug release characteristics were investigated using two groups of factors. In contrast to the drug entrapment and conductivity of the niosomal suspension, the vesicular size parameters as well as the zeta potential were inversely proportional with the homogenization parameters. Drug release was significantly affected by the compositional factors rather than the physical ones. The current study demonstrated the usefulness of the microfluidization for the production and further scale-up of anti-HIV niosomes with very small mean vesicular sizes.

Ismail Mouzam *et al.,* 2011, prepared formulations were then subjected to the drug and excipients used. The method adopted achieved higher entrapment efficiency of 95% with least concentration of span 60 and cholesterol, while vesicular diameter observed was 4.88micrometer

for the sane concentration. The TEM images showed the formulated niosomes were spherical and discrete. At equimolar concentration span 60 and cholesterol (1:1) higher invitro release of 98.53% was observed, and was considered to be best formulation.

Malay K Das *et al.*, 2011, developed the +sorbitan ester niosomes for topical delivery of rofecoxib. Niosomes were prepared by thin film method and were analyzed for size, entrapment efficiency and drug retention capacity. Niosomal vesicles were then incorporated into blank Carbopol gel to form niosomal gel. The size and entrapment if the niosomal vesicles increased with gradual increase in HLB value of non ionic surfactants used. The niosomal gel showed a prolong drug release behavior compared to plain drug gel.

Mohamed Nasr *et al.*, 2010, In Vitro and In Vivo Evaluation of Proniosomes Containing Celecoxib for Oral Administration. Proniosomes were prepared by sequential spraying method, which consisted of cholesterol, span 60, and dicetyl phosphate in a molar ratio of 1:1: 0.1, respectively. The average entrapment percent of celecoxib proniosome-derived niosomes was about 95%. The prepared proniosomes showed marked enhancement in the dissolution of celecoxib as compared to pure drug powder. The bioavailability of 200 mg single dose of both celecoxib proniosomal formulation and a conventional marketed celecoxib capsule was studied in human volunteers. The obtained results show that the proniosomal formulation significantly improved the extent of celecoxib absorption than conventional capsule. The mean relative bioavailability of the proniosomal formulation to the conventional capsule was172.06±0.14%. The mean Tmax for celecoxib was prolonged when given as proniosomal capsule. There was no significant difference between the values of K and t1/2 for both celecoxib preparations

Esmaeil Moazeni *et al.*, 2010, developed a niosome-encapsulated ciprofloxacin (CPFX) HCl formulation for pulmonary delivery, the feasibility of encapsulation of CPFX in niosomes, its

stability and nebulization capability was evaluated. Various combinations of nonionic surfactants with cholesterol were used to prepare the formulations. The in vitro deposition data of the niosomal formulations were examined using an Andersen cascade impactor. Formulations composed of Span 60 and Tween 60 in combination with 40 mol% of cholesterol exhibited high encapsulation efficacy and stability and also had fine particle fraction and nebulization efficiency of about 61.9%_1.0 and 77.9_2.8, respectively. Minimal inhibitory concentration of the niosomal CPFX against some pulmonary pathogens were lower than free CPFX. Using the MTT assay in human lung carcinoma cell line (A549), niosome-entrapped CPFX showed significantly lower cytotoxicity in comparison to the free drug. These results indicate that niosome can be used as a carrier for pulmonary delivery of CPFX via nebulization.

Aarti Jagtap *et al.,* 2010, developed the formulation and evaluation of niosome entrapped pentoxifylline using in vivo bronchodilatory activity in guinea pigs. The objective of this study is to formulate niosomes of pentoxifylline, characterize niosomes in terms of entrapment efficiency, particle size distribution, in vitro release and stability and investigate the bronchodilatory activity of plain and niosomal pentoxifylline in vivo guinea pigs. Pentoxifylline was entrapped in niosomes by lipid layer hydration method using span60, cholesterol and dicetyl phosphate. The entrapment efficiency of niosomes of pentoxifylline was determined by separating the entrapped drug from the free drug by centrifugation. The in vitro release profile of the drug from niosomes was carried out in phosphate buffer saline (pH 7.4). The stability of niosomes was assessed by storage at 4 ± 1^0 , 25 ± 1^0, 37 ± 1^0 and 45 ± 1^0 for one month. The plain (20, 40 and 80mg/kg) and niosomal pentoxifylline (5, 10, 20 and 40 mg/kg) was injected intraperitoneally to guinea pigs for evaluating bronchodilatory activity. The entrapment

efficiency of niosomes of pentoxifylline was found to be 9.26±1.96% giving a sustained release of drugs over a period of 24 h and better stability over the period of storage.

Kandasamy Rukmani *et al.,* 2010, developed a formulation and optimization of zidovudine niosomes. The objective of this study was to evaluate process-related variables like hydration and sonication time, rotation speed of evaporation flask, and the effects of charge-inducing agent and centrifugation on zidovudine entrapment and release from niosomes. Formulation of zidovudine niosomes was optimized by altering the proportions of Tween, Span and cholesterol. The effect of process–related variables like hydration time, sonication time, charge-inducing agent, centrifugation and rotational speed of evaporation flask on zidovudine entrapment and release from niosomes was evaluated. The effect of changes in osmotic shock and viscosity were also evaluated. Non-sonicated niosomes were in the size range of 2-3.5 μm and sonicated niosomes formulated with Tween 80 and dicetylphosphate (DCP) had a mean diameter of 801nm.Zidovudine niosomes formulated with Tween 80 entrapped high amounts of drug and the addition of DCP enhanced drug release for a longer time (88.72% over 12 h). The mechanism of release from Tween 80 formulation was the Fickian type and obeyed first-order release kinetics. Niosomes can be formulated by proper adjustment of process parameters to enhance zidovudine entrapment and sustainability of release.

Kandasamy Ruckmani *et al.,* 2010, studied the tissue distribution, pharmacokinetics and stability studies of zidovudine delivered by niosomes and proniosomes. Proniosomes were prepared in the form of a slurry using β-cyclodextrin as carrier. The effect of the surfactants tween and span and the negative charge inducers (DCP) on tissue distribution of niosomes and proniosomes was studied. The distribution of ZDV in lungs, kidney, heart, liver and spleen of mice after intravenous bolus injection was higher in tween 80 niosomes without DCP than either

niosomes with DCP or tween 80 proniosomes. The amount of ZDV in plasma was low in tween 80 niosomes without DCP. The results of a pharmacokinetic study in rabbits confirmed that tween 80 formulations with DCP were cleared from the circulation within five hours. An increased half-life of 202 minutes and mean residence time of 212.1 minutes was observed in tween 80 formulation. A stability study showed that after 90 days of storage, the drug leakage from tween 80 formulations stored at room temperature was significant ($p>0.001$) compared to niosomes stored at 4^0C . Encapsulation ZDV in proniosomes reduced drug leakage from vesicles stored at room temperature.

Pavala Rani *et al.,* 2010, developed a formulation and evaluation of rifampicin and gatifloxacin niosomes on logarithmic – cultures of *Mycobacterium tuberculosis*. Niosomes are vesicles mainly consisting of non-ionic surfactants that encloses and encompasses the drug molecules. Niosomes of rifampicin and gatifloxacin were prepared by lipid hydration technique using rotary flash evaporator. The prepared rifampicin and gatifloxacin niosomes showed a vesicle size in the range of 100-300nm, the entrapment efficiency were 73% and 70% respectively. The *in vitro* release study showed that 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes respectively. The bactericidal activities of the niosomal formulation were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis* which showed greater inhibition and reduced growth.

AjayB.Solanki *et al.,* 2010, optimized the composition of niosomes containing aceclofenac for transdermal application, with a view to improve permeation of drug during an extended period of time. Niosomes were prepared by thin film hydration technique. A 32 factorial design was utilized to study the effect of the molar ratio of drug to lipid ($X1$) and volume of hydration medium ($X2$) on percentage drug entrapment (PDE) and vesicle size. Selected batches of

niosomes were incorporated in to Carbopol gel matrix to prepare the niosomal gel formulations, which were evaluated for *in-vitro* release, skin permeation and *in vivo* studies. . Each of the prepared niosomal gel formulations significantly improved ($P<0.05$) cumulative amount of drug permeated, steady state transdermal flux and increase in paw thickness. This study demonstrates that niosomal gel formulations may offer promise as a transdermal delivery of aceclofenac to improve efficiency and better patient compliance**.**

Arora Rajnish *et al.,* 2010, evaluated the release studies of Ketoprofen niosomes formulation. Ketoprofen niosome were prepared by thin film hydration method technique using surfactant, cholesterol, dicetyl phosphate & drug mixture in different weight ratios. The prepared niosomes were characterized by various physicochemical parameters & evaluation of release studies of entrapped Ketoprofen in niosomes were carried out by UV Visible spectrophotometeric method.

Abdul Hasan Sathali *et al.,* 2010, studied the evaluation of transdermal targeted niosomal drug delivery of terbinafine hydrochloride niosomes of terbinafine hydrochloride were formulated by thin film hydration method using different ratios of non ionic surfactant (tween 20, 40, 60, and 80) and cholesterol with constant drug concentration. The prepared formulations were evaluated for its vesicle size (by AFM), entrapment efficiency (by dialysis method) *in vitro* release studies and antifungal activities. Increase in surfactant concentration, increased the entrapment efficiency (up to 84.92%) and the formulation with surfactant cholesterol ratio 2:1 in each group of surfactant showed good entrapment.

Anand kumar *et al.,* 2010, studied the developing and optimizing niosomal formulation of aceclofenac in order to improve its bioavailability. In evaluation study the effect of the varying composition of non ionic surfactant and cholesterol on the properties such as encapsulation efficiency, particle size and drug release were studied. Moreover, the release of the drug was also

modified and extended over a period of 72 h in all formulations. NSF-3 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation NSF-6 was evaluated through dialysis membrane to get the idea of drug release. The mechanism of dug release was governed by Peppas model.

Abdul Hasan Sathali *et al.*, 2010, Studied the drug loaded ethosomes had been prepared using phospholipid and ethanol, were optimized and characterized for entrapment efficiency, vesicular size, shape, invitro skin permeation, skin retention, drug-membrane component interaction and stability. The ethosomes formulation having 4%w/v of phospholipid and 40%v/v of ethanol (F16) showing the greatest entrapment efficiency (72.91±0.64%) with small particle size (251±23nm) was selected for further skin permeation studies. The skin permeation and skin retention studies were performed on ethosomes formulation, liposomal formulation (4%w/v of phospholipid without alcohol), hydro ethanolic drug solution and phosphate buffer saline (pH7.4) drug solution. Among them, ethosomal formulation showed higher cumulative percentage of drug permeation (60.37±5%) and more skin retention (619.60±18μg/cm2) after 12 hours than the other formulations. The ethosomal vesicles were incorporated in Carbopol gel base and its anti-inflammatory efficiency was compared with the marketed diclofenac gel. The pharmacodynamic studies showed the enhanced anti-inflammatory activity of ethosomal gel than the marketed gel formulation. Our results suggest that the ethosomes are an efficient carrier for dermal and transdermal delivery of diclofenac potassium.

Aranya Manosroi *et al.*, 2010, developed the gallidermin (Gdm) loaded in anionic niosomes composed of Tween 61/CHL/DP (1:1:0.05 molar ratio) gave the highest entrapment efficiency (45.06%). (25.74±5.05_gcm−2 h−1) in VED to the unloaded Gdm incorporated in gel. This study has suggested that Gdm loaded in anionic niosomes and incorporated in gel is the superior

topical antibacterial formulation because of the high accumulation in the skin with no risk of systemic effect.

Srikanth.K *et al.,* 2010, developed the formulation and evaluation of topical meloxicam niosomal gel niosomes were prepared by thin film hydration method using non-ionic surfactants, cholesterol and drug in different ratios. The prepared niosomes were characterized for size, shape, entrapment efficiency invitro drug release and in vivo performance. The niosomes appeared as round in shape and size range was found to be 1.54 – 2.64 micro meter. In vivo anti inflammatory activity of niosomal gel prepared carrageenam induced rat paw edema method. The studies were demonstrated that niosomal gel was shown better pharmacological activity than the conventional preparations.

Pratap S.Jadon *et al.,* 2009, enhanced oral bioavailability of griseofulvin via niosomes. Niosomes were prepared by using different nonionic surfactants span 20, span 40, and span 60. The lipid mixture consisted of surfactant, cholesterol, and dicetyl phosphate in the molar ratio of 125:25:1.5, 100:50:1.5, and 75:75:1.5, respectively. The niosomal formulations were prepared by thin film method and ether injection method. The influence of different formulation variables such as surfactant type, surfactant concentration, and cholesterol concentration was optimized for size distribution and entrapment efficiency for both methods. Result indicated that the niosomes prepared by thin film method with span 60 provided higher entrapment efficiency. The niosomal formulation exhibited significantly retarded in vitro release as compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability of griseofulvin in albino rats after a single oral dose. The maximum concentration (Cmax) achieved in case of niosomal formulation was approximately double (2.98 μg/ml) as compared to free drug (1.54 μg/ml). Plasma drug profile also suggested that the developed niosomal system

also has the potential of maintaining therapeutic level of griseofulvin for a longer period of time as compared to free griseofulvin. The niosomal formulation showed significant increase in area under the curve0-24 (AUC; 41.56 µg/ml h) as compared to free griseofulvin (22.36 µg/ml h) reflecting sustained release characteristics. In conclusion, the niosomal formulation could be one of the promising delivery system for griseofulvin with improved oral bioavailability and prolonged drug release profiles.

Rathi Jagdish Chandra *et al.,* 2009, developed the formulation and evaluation of maltodextrin based proniosomes loaded with indomethacin. Microscopy confirms that all particles are uniform in size and shape. The entrapment efficiency was determined by separating the unentrapped using dialysis. The positive values of zeta potential indicated that the indomethacin niosomes were stabilized by electrostatic repulsion forces..In the stability it was observed that the drug leakage from the vesicles was least at 4º followed by 25ºC.

Shyamala Bhaskaran *et al.,* 2009, studied the comparative evaluation of niosome formulations prepared by different techniques. Niosomes containing salbutamol sulphate was prepared using Span 60 as the surfactant, by employing different techniques namely, thin film hydration, hand shaking, ether injection, lipid layer hydration and trans membrane pH gradient method. The drug encapsulation efficiency varied from 62 % to 87 %. *In vitro* drug release studies was carried out and formulation exhibited retarded release for 24 h. Transmembrane pH gradient method was found to be most satisfactory which released 78.4 % of drug in 24 h. This formulation was lyophilized and characterized by infrared spectroscopy. Tissue distribution studies in albino rats and bio- availability studies in rabbits were carried out.

Vijay Prakash pandey *et al.,* 2009, developed the preparation and characterization non-ionic surfactant vesicles for ophthalmic use. In the present study, the nonionic surfactant vesicles were

prepared by lipid film hydration method using span 60 and cholesterol with various molar ratios and characterized for entrapment efficiency; in-vitro drug release, surface charge, rheological character, physical stability, minimum inhibitory concentration, in-vivo drug release and ocular irritation were conducted. The span 60: cholesterol in molar ratio of 100:60 showed higher entrapment of drug and released73.77 % at 10th h and the availability of drug in the aqueous humor was 4.373µg/ml (Cmax), confirmed by HPLC method. The histopathology study also confirmed the safe use of niosomes. Study may be concluded that the non-ionic surfactant vesicles formulated with span 60 and cholesterol in a molar ratio of 100:60 showed potential approach to improve the ocular bioavailability of ofloxacin for the prolonged period of time.

Jong Soo Woo *et al.,* 2009, studied the formulation and invitro assessment of minoxidil niosomes for enhanced skin delivery. Niosomes formed from polyoxyethylene alkyl ethers (BrijTM) or sorbitan monoesters (SpanTM) with cholesterol molar ratios of0, 1 and 1.5 were prepared with varying drug amount 20–50mg using thin film-hydration method. The prepared systems were characterized for entrapment efficiency, particle size, zeta potential and stability. Skin permeation studies were performed using static vertical diffusion Franz cells and hairless mouse skin treated with either niosomes, control minoxidil solution (propylene glycol–water–ethanol at 20:30:50,v/v/v) or a leading topical minoxidil commercial formulation (Minoxyl). The results showed that the type of surfactant, cholesterol and incorporated amount of drug altered the entrapment efficiency of niosomes. Higher entrapment efficiency was obtained with the niosomes prepared from Span 60 and cholesterol at1:1 molar ratio using 25mg drug. Niosomal formulations have shown a fairly high retention of minoxidil inside the vesicles (80%) at refrigerated temperature up to a period of 3 months. It was observed that both dialyzed and non-dialyzed niosomal formulations (1.03±0.18 to 19.41±4.04%) enhanced the percentage of

dose accumulated in the skin compared to commercial and control formulations (0.11±0.03to 0.48±0.17%) except dialyzed Span 60 niosomes. The greatest skin accumulation was always obtained with non-dialyzed vesicular formulations.

`**Praveen S. Hiremath *et al.*, 2009,** prepared a proliposomes of exemestane for improved oral delivery: Formulation and invitro evaluation using PAMPA, Caco-2 and rat intestine..The present study was to develop proliposomes formulations to enhance the oral bioavailability of exemestane by improving solubility, dissolution and / or intestinal permeability. Proliposomal powder formulations were prepared using different ratio of drug and lipids, cholesterol by solvent evaporation method. The effect of phospholipids compostion and drug: lipids ratio on invitro performance of proliposomes was studied. Proliposomes were characterized for the particle size distribution, thermal characteristics by the DSC and dissolution behaviour. Further, the formulated proliposomes were subjected to invitro permeation or transport studies using different models such as rat intestine, parallel artificial membrane permeability assay and Cac-2 cell line. Proliposomes provided enhanced exemestane dissolution due to incorporation into the phospholipid bilayers and change in the physical state from crystalline to amorphous. The invitro transport studies in rat intestine, PAMPA and cac-2 models revealed that the proliposomes were successful in enhancing the permeation exemestane.

Meenakshi Chauhan *et al.*, 2009, developed the span-60 niosomal oral suspension of fluconazoloe: formulation and *in vitro* evaluation. Different batches of Fluconazole niosomal preparations were prepared by changing the surfactant concentration but keeping the cholesterol concentration constant. The surfactant used was Span 60 and the five batches of niosomal preparations prepared were in the ratios 1:1:1, 1.5:1:1, 2:1:1, 2.5:1:1 and 3:1:1 (surfactant:

cholesterol: drug). Furthermore, the release profile, entrapment efficiency, size distribution and stability of these niosomes under various temperatures were studied.

Mahmoud Mokhtar *et al.*, 2008, studied the proniosomal gels or solutions of flurbiprofen were developed based on span 20 (Sp 20), span 40 (Sp 40), span 60 (Sp 60), and span 80 (Sp 80) without and with cholesterol. Nonionic surfactant vesicles (niosomes) formed immediately upon hydrating proniosomal formulae. The entrapment efficiency (EE %) of flurbiprofen (a poorly soluble drug) was either determined by exhaustive dialysis of freshly prepared niosomes or centrifugation of freeze-thawed vesicles. Results indicated that the EE% followed the trend Sp 60 (C18)>Sp 40 (C16)>Sp 20 (C12)>Sp 80 (C18). Cholesterol increased or decreased the EE% depending on either the type of the surfactant or its concentration within the formulae. The maximum loading efficiency was 94.61% when the hydrating medium was adjusted to pH 5.5. Increasing total lipid or drug concentration also increased the EE% of flurbiprofen into niosomes. However, incorporation of either dicetyl phosphate (DCP) which induces negative charge or stearyl amine (SA) which induces positive charge decreased the EE% of flurbiprofen into niosomal vesicles. Finally, in vitro release data for niosomes of Sp 40 and Sp 60 showed that the release profiles of flurbiprofen from niosomes of different cholesterol contents is an apparently biphasic release process.

Varaporn Buraphacheep Junyaprasert *et al.*, 2008, studied the effect of charged and non-ionic membarane additives on physic chemical properties and stability of niosomes**.** The niosomes were composed of 1:1 mole ratio of Span 60: cholesterol as vesicle forming agents. The results show that incorporation of salicylic acid to the niosomes did not affect zeta potential values; however, addition of the membrane additives changed the zeta potential depending on the type of the additives. Transmission electron microscopy revealed that niosomes had unilamellar

structure. The particle sizes of all developed niosomes were between 217 to360 nm. The entrapment efficiency (%E.E.) of all salicylic acid niosomes at pH 3 was higher than that of niosomes at pH 5, indicating that salicylic acid in unionized form was preferably incorporated in niosomes. Furthermore, the positively charged niosomes showed the highest %E.E. of salicylic acid owing to electrostatic attraction between STR and salicylic acid. After 3 months of storage at 4°C, the particle size of the niosomes remained in the nano size range except for DCP salicylic acid niosomes at pH 3 whose size increased due to an instability of DCP at low pH. In addition, all niosomes showed no leakage of the salicylic acid after 3 months of storage indicating the good stability.

Ghada Abdelbary *et al.*, 2008, developed a niosome- encapsulated gentamicin for ophthalmic controlled delivery. Niosomal formulations were prepared using various surfactants (Tween60, Tween 80 or Brij 35), in the presence of cholesterol and a negative charge inducer dicetyl phosphate (DCP) in different molar ratios and by employing a thin film hydration technique. The ability of these vesicles to entrap the studied drug was evaluated by determining the entrapment efficiency %EE after centrifugation and separation of the formed vesicles. Photomicroscopy and transmission electron microscopy as well as particle size analysis were used to study the formation, morphology and size of the drug loaded niosomes. Results showed a substantial change in the release rate and an alteration in the %EE of gentamicin sulphate from niosomal formulations upon varying type of surfactant, cholesterol content and presence or absence of DCP. In-vitro drug release results confirmed that niosomal formulations have exhibited a high retention of gentamicin sulphate inside the vesicles such that their invitro release was slower compared to the drug solution. A preparation with 1:1:0.1 molar ratio of Tween60, cholesterol and DCP gave the most advantageous entrapment (92.02%±1.43) and release results

(Q8h=66.29%±1.33) as compared to other compositions. Ocular irritancy test performed on albino

Biswal.S *et al.,* 2008, studied the vesicles of non-ionic surfactants (Niosomes) and drug delivery potential. Niosomes exhibit more chemical stability than liposomes (a phospholipids vesicle) as non-ionic surfactants are more stable than phospholipids. Non-ionic surfactants used in formation of niosomes are polyglyceryl alkyl ether, glucosyldialkyl ether, crown ether, polyoxyethylenealkyl ether, ester-linked surfactants, and steroid-linked surfactants and a spans, and tweens series. Niosomes preparation is affected by processes variables, nature of surfactants, and presence of membrane additives and nature of drug to be encapsulated.

Massimo Fresta *et al.,* 2008, prepared a innovative bola- surfactant as topical delivery systems of 5- fluorouracil for the treatment of skin cancer .An innovative niosomal system made up of _hexadecyl-bis-(1-aza-18-crown-6) (Bola), Span 80® and cholesterol (2:5:2 molar ratio) was proposed as a topical delivery system for 5-fluorouracil (5-FU), largely used in the treatment of different forms of skin cancers. Bola-niosomes showed a mean size of 400 nm, which were reduced to 200 nm by a sonication procedure with a polydispersion index value of 0.1. Bola niosomes showed a loading capacity of □40% with respect to the amount of 5-FU added during the preparation. 5-FU-loaded bola-niosomes were tested on SKMEL-28 (human melanoma) and HaCaT (non-melanoma skin cancer with specific mutations in the p53 tumor suppressor gene) to assess the cytotoxic activity with respect to the free drug. 5-FU-loaded bola-niosomes showed an improvement of the cytotoxic effect with respect to the free drug. Confocol laser scanning microscopy studies were carried out to evaluate both the extent and the time-dependent bola-niosome–cell interaction. The percutaneous permeation of 5-FU-loaded niosomes was evaluated by using human stratum corneum and epidermis membranes. Bola-niosomes provided an

increase of the drug penetration of 8- and 4-folds with respect to a drug aqueous solution and to a mixture of empty Bola-niosomes with a drug aqueous solution.

Manivannan Rangasamy *et al.,* 2008, evaluated the acyclovir entrapped niosomes were prepared by hand shaking and ether injection process with different ratios of (1:1, 1; 2 and 1:3) cholesterol (CHOL) and Span-80 (Non-ionic surfactant).The niosomes prepared were in the size range of 0.5- 5 microns in the case of hand shaking process and 0.5-2.5 microns in the case of Ether injection process. The order of encapsulation efficiency increases when span-80 concentration was increased. In-vitro release study on acyclovir niosomes indicates 76.64% release for formulation prepared with CHOL: Span-80 (1:1) and it takes an extended period of 1 day and 16 h for release.

Ismail A.Attia *et al.,* 2007, studied the influence of a niosomal formulation on the oral bioavailability of acyclovir in rabbits. The purpose of this research was to prepare acyclovir niosomes in a trial to improve its poor and variable oral bioavailability. The nonionic surfactant vesicles were prepared by the conventional thin film hydration method. The lipid mixture consisted of cholesterol, span 60, and dicetyl phosphate in the molar ratio of 65:60:5, respectively. The percentage entrapment was ~11% of acyclovir used in the hydration process. The vesicles have an average size of 0.95 μm, a most probable size of 0.8 μm, and a size range of 0.4 to 2.2 μm. Most of the niosomes have unilamellar spherical shape. Invitro drug release profile was found to follow Higuchi's equation for free and niosomal drug. The niosomal formulation exhibited significantly retarded release compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability of acyclovir in rabbits after a single oral dose of 40 mg kg−1. The average relative bioavailability of the drug from the niosomal dispersion in relation to the free solution was 2.55 indicating more than 2-fold

increase in drug bioavailability. The niosomal dispersion showed significant increase in the mean residence time (MRT) of acyclovir reflecting sustained release characteristics.

Prasun Bandyopadhyay *et al.,* 2007, evidence for vesicle formation from 1:1 non ionic surfactant span 60 and fatty alcohol mixtures in aqueous ethanol: potential delivery vehicle composition. A study of the self-organization of non ionic surfactant span 60 in presence of fatty alcohol (stearyl, cetyl and lauryl) is presented. When ethanolic solution of the surfactant-fatty alcohol (1:1) mixture is added in water spontaneous large unilamellar vesicles are formed which may potentially be useful vehicles for drug delivery purposes. Vesicular suspension has been characterized by transmission electron microscopy, dynamic light scattering, and confocol laser scanning microscopy, dye entrapment and release studies. Surface tension measurement indicates the suitability of fatty alcohols towards spontaneous vesicle formation from span 60.

Tianqing liu *et al.,* 2007, preparation and properties of highly stable innocuous niosome in Span 80/PEG 400/H2O system .The highly stable innocuous niosome composed of only three components is successfully prepared in Span 80/PEG 400/H2O system. The niosome properties are studied by some means of freeze fracture replication-transmission electron microscopy, negative staining-transmission electron microscopy, dynamic light scattering and differential scanning calorimetry. The obtained results indicate that the niosome can be stable for over one year. The niosome diameter is between 100 and 180 nm. The compositions of the system affect the preparation and properties of the niosome. But the temperature and ionic intensity do not distinctly change the stability and the radius.

Majid Tabbakhian *et al.,* 2006, studied the enhancement of follicular delivery of finasteride by liposomes and niosomes, invitro permeation and in vivo deposition studies using hamster flank and ear models. Vesicles were prepared by the thin film hydration technique and characterized

with regard to the size, drug entrapment effiency and gel-liquid transition temperature. Invitro permeation of finasteride through hamster flank skin was showed that liquids .

Anna M.Fadda *et al.*, 2006, developed the niosomes as carriers for tretinoin III.A study into the in vitro cutaneous delivery of vesicle- incorporated tretinoin. Positively and negatively charged vesicular formulations were prepared using either stearylmine or dicetylphosphate as a charge inducer. Niosomes made with polyoxyethylene lauryl ether and liposomes made with soy phosphatidylcholine were also prepared and studied. Vesicular formulations were characterized by transmission electron microscopy and optical and light polarized microscopy for vesicle formation and morphology, and by dynamic laser light scattering for size distribution. The effect of the vesicular incorporation of tretinoin on its transdermal delivery through the newborn pig skin was also investigated invitro using Franz cells, in comparison with a commercial formulation of the drug. The amount of tretinoin delivered through and accumulated in the several skin layers was detected by HPLC. The results showed that tretinoin cutaneous delivery is strongly affected by vesicles composition and thermodynamic activity of the drug. Negatively charged niosomal formulations, which are saturated with tretinoin, have shown to give higher cutaneous drug retention than both liposomes and commercial formulation

Vyas.S.P *et al.*, 2006, developed the niosomal system for delivery of rifampicin to lymphatics. Niosomes containing rifampicin were prepared using various non ionic surfactants of sorbitan ester class and cholesterol in 50:50 percent mole fraction ratio. The drug- entrapment vesicles were characterized for their shape, size, drug entrapment efficiency and in vitro release rare. On the basis of in vitro characterization, the niosomes showing maximum entrapment and minimum release rate were selected for in vivo evaluation.

Behrooz Nasseri *et al.,* 2005, studied the effect of cholesterol and temperature on the elastic properties of niosomal membranes. The mechanical characteristics of non-ionic bilayer membranes composed of sorbitan monostearate, cholesterol and poly-24-oxyethylene cholesteryl were studied by measuring the modulus of surface elasticity (μ), a measure of membrane strength, as a function of cholesterol content and temperature. The modulus of surface elasticity increased slowly with increasing cholesterol concentration, with a sharp increase around 40 mol% cholesterol (on average an increment of 0.43×106 Nm−2 per molar percentage), and displayed a maximum of 6.5×106 Nm−2 around 47.5 mol% cholesterol. Further cholesterol resulted in a decrease in μ. Generally the interaction of cholesterol with the sorbitan monostearate should increase the rigidity of the membrane. However, the latter effect may be due to the formation of cholesterol clusters at high cholesterol content where excess amounts of cholesterol cannot interact with the sorbitan monostearate, and deposits on the bilayers compromising their uniformity, strength and permeability. This behaviour was evident when measurements were carried out above and below 25 ◦C.

Vyas.S.P *et al.,* 2005, developed the non ionic surfactant based vesicles (niosomes) for non-invasive topical genetic immunization against hepatitis B. Niosomes composed of span 85 and cholesterol as constitute lipids were prepared by reverse phase evaporation method. Prepared niosomes were characterized for their size, shape and entrapment efficiency. The immune stimulating activity was studied by measuring serum anti-HBs Ag titer and cytokines level following topical application of niosomes in Balb/c mice and results were compared with naked DNA and liposomes encapsulated DNA applied topically as well as naked DNA and pure recombinant HBsAg administered intramuscularly. It was observed that topical niosomes elicited a comparable serum antibody titer and endogenous cytokines levels as compared to

intramuscular recombinant HBsAg and topical liposomes. The study signifies the potential of niosomes as DNA vaccine carriers for effective topical immunization.

Samar Mansour *et al.,* 2005, studied the preparation and evaluation of reverse – phase evaporation and multilayer niosomes as ophthalmic carriers of acetazolamide. Niosomes formed from span 40 and span 60 and cholesterol in the molar ratio of 7:4, 7:6 and 7:7 were prepared using reverse-phase evaporation and thin film hydration methods. The prepared systems were characterized for entrapment efficiency, size, shape and in vitro release. Stability studies were carried out to investigate the leaching of drug from niosomes during storage. The intraocular pressure lowering activity of acetazolamide niosomal formulations in rabbits was measured using tonometer. The results showed that the type of surfactant, cholesterol content and the method of preparation altered the entrapment efficiency and drug release rate from niosomes. Higher entrapment efficiency was obtained with multilayer niosomes prepared from Span60 and cholesterol in a 7:6 molar ratio. Niosomal formulations have shown a fairly high retension of acetazolamide inside the vesicles at a refrigerated temperature up to period of 3 months. Multilamellar acetazolamide niosomes formulated with Span 60 and cholesterol in a 7:4 molar ratio were found to be most effective and showed prolonged decrease in IOP.

Suresh P.Vyas *et al.,* 2005, developed the non-invasive vaccine delivery in transfersomes, niosomes and liposomes: a comparative study. Niosomes and liposomes were prepared and characterized for shape, shape and entrapment efficiency. These vesicles were extruded through polycarbonate filter to assess the elasticity of the vesicles. The stimulating activity of transfersomes, niosomes and liposomes were studied by measuring the serum anti-TT IgG titre following topical immunization. The immune response elicited by topical immunization was compared with that elicited by same dose of alum-adsorbed tetanus toxoid given intramuscularly.

The results indicate that optimal formulations of transfersomes, niosomes and liposomes could entrap 72.7±3.4, 42.5±2.4 and 41.3±22% of antigen and their elasticity values were 124.4±4.2,29.3±2.4 and 21.7±1.9, respectively.

Mullaicharam.A.R *et al.,* 2004, developed the formulation, optimization and stability of rifampicin niosomes. A niosomal drug delivery system of rifampicin was developed using factorial design and the niosomes were optimized. The effects of alteration of process variables like volume of solvent, hydration time, volume of hydration medium and sonication time were studied. The prepared niosomes were characterized for size, shape and lamellarity. The stability of niosomes in terms of retention of drug was measured at refrigerated temperature and ambient temperature (25-35^{o}C) for the period of 60 days.

Ibrahim A.Alsarra *et al.,* 2005, niosomes have been utilized as carriers to enhance Atenolol absorption from the gastrointestinal tract. The prepared niosomes were evaluated for enhancement effect on drug permeation across the intestinal membranes using an everted sac technique. Permeation through an everted intestinal sac showed a significant enhancement effect (more than 4 fold).

Ambikanandan Misra *et al.,* 2002, niosomes were prepared by lipid film hydration method using tweens and spans. Preparation of niosomes was optimized for highest percent drug entrapment. The prepared niosomes were incorporated into 1 percent Carbopol gel base evaluated for drug diffusion across human cadaver skin using modified validated diffusion cell. The drug retension studies in niosomes were performed at refrigerated temperature and at room temperature for the period of 2 months. Invivo performance of plain drug gel, niosomal entrapped drug in Carbopol gel base and marketed formulation were evaluated using acute rat

paw edema mehod. High mean percentage edema inhibition was observed for niosomal nimesulide gel after 24 hours.

PATENTS ON NIOSOMES IN VARIOUS UNIVERSITIES

- Albert Einstein College of Medicine, New Hyde Park. Ain Shams University, Cairo Centre for Drug Delivery Research Drug Delivery Research.
- The School of Pharmacy, university of London.
- St.johns university and Dept. of pharmaceutical Sciences.
- Vicks Research Center, Richardson, Vicks Inc., Shelton.
- University of Strath Clatde.
- University of Wro Claw.
- 7. University of Witten Germany, School of Medicine.
- University of California.
- University of Pittspurgh.
- Department of Chemistry, University of Oxford.
- Department of Pharmaceutical Engineering, Koyang University, Nonsan. Korea.
- Technical University, Clausthal. Russia.
- Institute for Nuclear Research, Dubna, Russia.
- Australia national University, Canberra ACT, Australia.
- University of Kentucky Medical Center, Lexington.
- University of Waterloo, Waterloo.

Patent Status

1. US Patent Number: 6576254

Ucheghu IF, Department of Pharmaceutical Sciences.

The School of Pharmacy. University of London.

2. US Patent Number: 5910488

Alejandro Madrigal, Stand ford University.

3. US Patent application: 0040115278

Oxford University, Newyork.

4. US Patent application: 2695331996

Ucheghu IF, Department of Pharmaceutical Sciences.

5. US Patent application: 2106913146

Bouwsta.J. The Technical University of Munich.Ismanigersh.

CHAPTER-VI

SCOPE OF WORK

Nonsteroidal anti-inflammatory agents (NSAIDs) are class of drugs which budded from the bark of willow in the mid-eighteenth century. Now a day, there has been a rapid increase in the number of products that have been designed to deliver NSAIDs. These include creams, gels, ointment, and more complex transdermal systems. A number of approaches have been continuously investigated so as to enhance dermal delivery by use of prodrugs, ultrasound, ionotophorosis and microneedles. But the choice of the most appropriate drug depends on a number of factors which includes its potency, its ability to permeate the stratum corneum, its lack of local skin toxicity and stability towards metabolizing enzymes present on the skin surface.

Etoricoxib is one of the NSAID widely used for musculoskeletal complaints especially arthritis, rheumatoid arthritis, Osteo arthritis, dental pain and gouty attacks.

Oral dose of etoricoxib can causes an increased risk of serious gastrointestinal adverse events including bleeding, ulceration and perforation of the stomach or intestines which can be fatal. These events can occur at any time during use and without warning symptoms. This drug may also cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction and stroke.

Due to presence of these oral adverse effects, necessitates the need for investigating other routes of drug delivery of Etoricoxib. Transdermal delivery of the drug can improve its bio activity and transdermal effect, reduce the side effects, sustained effect and enhance therapeutic efficacy. This can be achieved only when the drug has entered the lower layers of the skin, then

only it can be absorbed by blood and transported to the site of action, or penetrate deeper in to areas where inflammation occurs. Pure drug or liposomal formulations not reach the lower layers of the skin.

Niosomes a novel liposome, is especially suitable for topical and transdermal administration carrier. Compared to other liposomes, the physical and chemical properties of niosomes make the delivery of the drug through the stratum corneum in to a deeper layer efficiently or even into the blood circulation. Etoricoxib is a hydrophobic drug generally the entrapment efficiency of the niosomes of a water insoluble drug is higher than that of the other vesicle formulation. So the etoricoxib niosomal gel formulation may be better than other transdermal or topical formulation of NSAIDS.

CHAPTER-VII

PLAN OF WORK

1. Standard Curve for Etoricoxib
2. Pre Formulation Studies

    FT-IR Studies

    DSC Studies

3. Formulation of Etoricoxib Niosomes

    Drug Content

    Entrapment Efficiency

    invitro Release Studies

    invitro Release Kinetics

4. Formulation of Etoricoxib niosomal gel
5. Evaluation of the Prepared Etoricoxib Niosomal Gel

    Drug Content
    pH Measurements
    Rheology Studies
    Particle Size
    Zeta Potential
    Transmission Electron Microscopy
    invitro Release Studies
    . *invitro Release Kinetics*
    invivo-Anti-Inflammatory Studies
    Stability Studies
    Analysis of variance

CHAPTER-VIII

MATERIALS

• Drug	:	Etoricoxib
• Cholesterol	:	S.D.Fine Chem. Ltd., India
• Sorbiton manolaurate	:	Loba Chemie, India
• Sorbiton monopalmitate	:	Loba Chemie, India
• Sorbiton monostearate	:	Loba Chemie, India
• Sorbiton monooleate	:	Loba Chemie, India
• Tween 60	:	Loba Chemie, India
• Tween 80	:	Loba Chemie,India
• Brij-52	:	Loba Chemie,India
• Chloroform	:	Rankem, India
• Methanol	:	Rankem, India
• n-propanol	:	Nice Chemicals, India
• Sodium chloride	:	Central Drug House, India
• Potassium dihydrogen ortho phosphate	:	Nice Chemicals, India
• Disodium hydrogen ortho phosphate	:	Qualigens, India
• Dialysis membrane	:	Himedia, India

EQUIPMENT USED

• Rotary Flash Evaporator	:	Super fit rotary flash evaporator, India
• Ultra Sonicator	:	Vibronic's Ultrasonic Processor, India
• Electronic Balance	:	A&D Company, Japan
• Magnetic Stirrer	:	MC Dalal & Co, India
• UV Visible Spectrophotometer	:	UV Pharma Spec 1700, Shimadzu, Japan
• Cooling Centrifuge Apparatus	:	Eppendorf Centrifuge 5417R, Germany
• Malvern zeta analyzer	:	Malvern Instruments, Uk
• FT-IR Spectrophotometer	:	Shimadzu, Japan
• Differential Scanning Calorimeter	:	Perkin Elmer STA 6000 Thermal Analyzer,USA
• Refrigerator	:	Kelvinator, India
• Environmental chamber	:	Inlab Equipments (Madras) Pvt. Ltd, India.
• Transmission electron microscopy	:	Technai Sprit, FEI, Netherlands
• Digital Pletheesmometer	:	Ugobasile, Italy

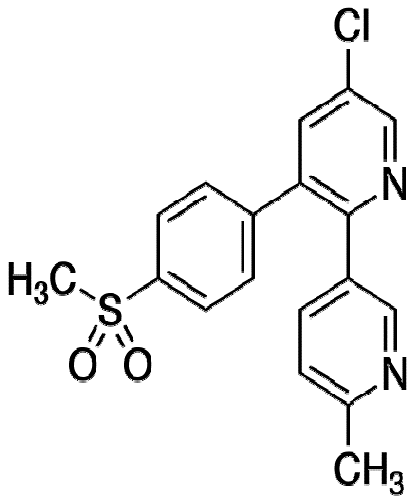
CHAPTER-IX

DRUG PROFILE

DRUG NAME : **ETORICOXIB**

SYNONYMS : MK-663

CHEMICAL STRUTURE



CHEMICAL FORMULA : **C_{18} H_{15} CLN_2O_2S**

IUPAC NAME : 5- chloro-3- (4- methane sufonyl phenyl) 2- 6- methyl pyridin-3-yl) pyridine.

PHYSICAL CHEMICAL PROPERTIES:

- Molecular weight **:** **358.842**
- Physical state : SOLID
- Solubility : 3.28 e-0.3g/l
- Log p value : 3.70
- Refractivity : 95.04
- Polarizability : 36.42

CATEGORIES

Cyclo-oxignenase inhibitors

CLASSES

- Bipyridines
- Phenyl propenes
- Pyridines & Derivatives
- Sulfonyls
- Benzene Derivatives.

PHARMACODYNAMICS;

Etoricoxib selectively inhibits isoform 2 of cyclo-oxignenase enzyme (COX-2). This reduces prosta glanding (PGs) generation from arachidonic acid, there by decrease the inflammation (www.drugbank.com).

PHARMACOKINETICS;

- *Absorbtion*

  Oral absorption

- *Metabolism*

  Cytochrome P450

- *Bioavailability*

  100% -ORALY

- *Protein binding*

  92%

- *Half-life*

  22 HOURS

THERAPEUTIC USES

- Acute gout
- Rheumatoid arthritis
- Osteo arthritis
- Chronic low back pain
- Ankylosing Spondylitis

SIDE EFFECTS

Very common side effects

- Ulceration, bleeding or perforation of the stomach or intestinal lining
- Increased risk of heart attacks and stroke.

Common side effects (affect between 1 in 10 & 1 in 100 people)

- Dizziness
- Head ache
- High blood pressure
- Cold or flu- like syndrome

Un common side effects (affect 1 in 100 and 1 in 1000 people)

- Change in appetite
- Anxiety or depression
- Change in taste
- Blurred vision
- Nose bleeds

USE WITH CAUTION IN

- Elderly people
- History of disorders affecting the Stomach or intestines.
- Decreased liver function
- Liver cirrhosis
- Dehydration
- History of heart failure
- Hypertension
- Diabetes
- Smokers

NOT BE USED IN

- Children and adolescent under 16 years of age.
- Severely decreased liver function
- Moderate to severely decreased kidney function
- Active peptic ulcer or bleeding from the gut.
- Heart failure
- Pregnancy
- Breast feeding

Available brands of Etoricoxib

- Coxet (Anthus)
- Ebov (Glenmark (Integrace))
- Erofica (Micro HC)

- Eteron (CFL)
- Eticox (East West)
- Etody (AHPL)
- Etofan (Emar)
- Etom (Intra Labs)
- Etorica (Micro Eros)
- Etoshine (Sun)
- Etoxib (Unichem)
- Etozox (Cipla)
- ETRO (Allenge)
- Etrobax (Ranbaxy)
- Hicox (Systopic)
- Hireto (Aamorb (St.Morison))
- Ifydrox (Sanify (Syntonic))
- Kingcox (Cadila HC)
- Kretos (Glenmark (Majesta))
- L-KON (Laksun)
- M-KON (Madhav Biotech)
- Nucoxia (Zy. Cadila)
- Nucoxia -P (Zy. Cadila)
- Nucoxia -SP (Zy. Cadila)
- Retoz (Dr. Reddy's)
- Torcoxia (Torrent)

- Torcoxia BCD (Etoricoxib (with Betacyclodextrin) (Torrent)
- Xibra (Khandelwal)

DOSAGE

- Osteoarthritis – 60mg – once daily
- Rheumatoid – 90 mg – once daily
- Acute gout – 120mg – once daily

CHAPTER-X

EXCIPIENTS PROFILE

CHOLESTEROL

SYNONYM

Cholesterin, Cholesterolum

CHEMICAL NAME

Cholest -5- en-3β -ol.

EMPIRICAL FORMULA

$C_{17}H_{46}O$

MOLECULAR WEIGHT

386.67

FUNCTIONAL CATEGORY

- Emollient
- Emulsifying agent

DESCRIPTION

- Cholesterol occurs as white or faintly yellow, almost odourless, pearly leaflets, needles, powder or granules.
- On prolonged exposure to light and air, it acquires a yellow to tan color.

PROPERTIES

Boiling Point	-	360 ^{o}C
Density	-	1.052g/cm^3 for anhydrous form
Melting Point	-	147-150^{o}C
Solubility	-	Soluble in acetone and vegetable oils. Practically insoluble in water and chloroform

STABILITY AND STORAGE CONDITIONS

It is stable, and should be stored in a well-closed container and protected from light.

SAFETY

It is generally regarded as an essentially non-toxic and non-irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

Rubber or plastic gloves, eye protection and a respirator are recommended.

METHOD OF MANUFACTURE

The commercial material is normally obtained from the spinal cord of cattle by extraction with petroleum ether, but it may also be obtained from wool fat. Purification is normally accomplished by repeated bromination. Cholesterol may also be produced by entirely synthetic means.

REGULATORY STATUS

Induced in the FDA inactive ingredients.

SORBITAN MONOLAURATE

SYNONYM

Arlacel 20; Crill 1; Liposorb L; Montane 20; Sorbitan laurate; Span 20.

CHEMICAL NAME

Sorbitan mono dodecanoate.

EMPIRICAL FORMULA

$C_{18}\ H_{34}\ O_6$

MOLECULAR WEIGHT

346

DESCRIPTION

Yellow viscous liquid.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan (1,4-sorbitan), which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	:	≤7
Hydroxyl value	:	159-169
Saponification value	:	159-169
Density	:	1.01g/cm^3
HLB Value	:	8.6

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant

- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOPALMITATE

SYNONYMS

Ablunol S-40, Armotan MP, Liposorb P, Span 40, Arlacel 40, Montane 40, Sorbitan Palmitate.

CHEMICAL NAME

Sorbitan monohexa decanoate.

EMPIRICAL FORMULA

$C_{22}H_{42}$ O6

MOLECULAR WEIGHT

403

DESCRIPTION

It occurs as cream solid with a distinctive odour and taste.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexitan (1, 4 Sorbitan) which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	3 to 7
Hydroxyl value	270 to 303
Iodine valure	≤ 1
Density (g/cm^3)	1.0
HLB Value	6.7
Melting point	43^0C - 48^oC
Solubility	Soluble in oils and in most organic solvents. Insouble but dispersible in water.

FUNCTIONAL CATEGORY

- Emulsifying agent.
- Non ionic Surfactant.
- Solubilizing agent.
- Wetting agent.

STABILITY

It should be stored in a well-closed container in a cool, dry place.

SAFETY

It is generally regarded as non-toxic and non-irritant material.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOSTEARATE

SYNONYMS

Ablunol S-60, Alkamuls SMS, Sorgen 50, Tego SMS, Span 60, Arlacel 60, Durtan 60, Montane 60, Sorbitan Stearate.

CHEMICAL NAME

Sorbitan mono – Octadecanoate.

EMPIRICAL FORMULA

$C_{24}\ H_{46}\ O_6$

MOLECULAR WEIGHT

431

DESCRIPTION

It occurs as a cream solid with a distinctive odour and taste.

METHOD OF MANUFACTURE

Sorbitol is dehydrated to form a hexiton (1,4 Sorbitan) which is then esterified with the desired fatty acid.

PROPERTIES

Acid value	5 to 10
Hydroxyl value	235 to 260
Iodine value	≤ 1
HLB Value	4.7
Melting Point	$53^0C – 57^oC$
Solubility	Soluble in oils and in most organic solvents. Insoluble but dispersible In water.

FUNCTIONAL CATEGORY

- Emulsifying agent.
- Nonionic Surfactant.
- Solubilizing agent.
- Wetting agent.

STABILITY

- Gradual Soap formation occurs with strong acids or bases.
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

- It is generally regarded as non-toxic and non-irritant material.
- Very mildly toxic by ingestion.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

POLYSORBATE 60

SYNONYM

Atlas 70k, Atlas armotan PMS 20, Glycosporse s-20, Tween 60, Tween 60k, Tween 60VS.

CHEMICAL NAME

Sorbitanmono Octadecanoate.

EMPIRICAL FORMULA

$C_{64}\ H_{126}\ O_{26}$

MOLECULAR WEIGHT

1312

DESCRIPTION

Yellow oily liquid.

MEYHOD OF MANUFACTURE

Polysorbate are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbitol anhydride). The sorbitan is then partially esterified with a fatty acid such as oleic acid (or) stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbates.

PROPERTIES

Acid value	-	2.0
Hydroxyl value	-	81 – 96
Saponification value	-	45 - 55
Density (g/cm^3)	-	$1.1 g/cm^3$
HLB Value	-	14.9
Solubility	-	Soluble in ethanol and water. Insoluble in Mineral oil and vegetable oil.

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25mg/Kg body weight and moderately toxic by IV route.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

POLYSORBATE 80

SYNONYM

Atlas E, Capmul POE-o, Glycospere o-20,Tego SMO 80,Tego SMO 80 x, Tween 80.

CHEMICAL NAME

(Z) Sorbitan mono-9- Octadecanoate poly (oxy 1,2, ethanediyl) derivatives.

EMPIRICAL FORMULA

$C_{64}\ H_{124}\ O_{26}$

MOLECULAR WEIGHT

1310

DESCRIPTION

Yellow oily liquid.

MEYHOD OF MANUFACTURE

Polysorbate are prepared from sorbitol in a three-step process. Water is initially removed from the sorbitol to form a sorbitan (a cyclic sorbital anhydride). The sorbitan is then partially esterified with a fatty acid such as oleic acid (or) stearic acid to yield a hexiton ester. Finally, ethylene oxide is chemically added in the presence of a catalyst to yield the polysorbates

PROPERTIES

Acid value	-	2.0
Hydroxyl value	-	65 – 80
Saponification value	-	45 - 55
Density (g/cm^3)	-	$1.08 g/cm^3$
HLB Value	-	15
Solubility	-	Soluble in ethanol and water. Insoluble in mineral oil and vegetable oil.

FUNCTIONAL CATEGORY

- Emulsifying agent
- Nonionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing / Suspending agent.

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well-closed container in a cool, dry place.

SAFETY

- Daily intake according to the WHO limit is about 25mg/Kg body weight
- LD_{50} (Mouse, oral)-25g/Kg.

HANDLING PRECAUTIONS:

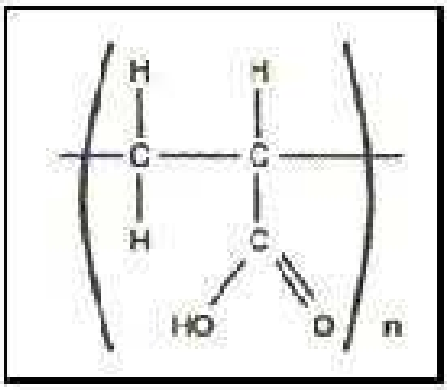
Eye protection and Gloves are recommended.

REGULATORY STATUS:

Polysorbates 20,40,60,65 and 80 are accepted as food additives in Europe. Polysorbates 20, 40, 60, and 80 are included in the FDA inactive ingredients guide (IM, IV, Oral, rectal, topical and vaginal preparations). Polysorbates are included in parenteral and non-parenteral medicines licensed in the UK.

CARBOPOL 940

Structure:



Synonyms

Acritamer acrylic acid polymer; Carbopol; carboxypolymethylene, polyacrylic acid; carboxyvinyl polymer; Pemulen;Ultrez. Chemical Name and CAS Registry Number

Carbomer

Empirical Formula and Molecular Weight

Carbomers are synthetic high-molecular-weight polymers of acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 56% and 68% of carboxylic acid (COOH) groups 104 400 g/mol for Carbopol 940 have been reported

Structural Formula

Carbomer polymers are formed from repeating units of acrylic acid. The monomer unit is shown above. The polymer chains are crosslinked with allyl sucrose or allylpentaerythritol.

Functional Category

Bioadhesive; emulsifying agent; release-modifying agent; suspending agent; tablet binder; viscosity-increasing agent.

Applications in Pharmaceutical Formulation or Technology

Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents.Formulations include creams, gels, and ointments for use in ophthalmic,(5–7) rectal,(8–10) and topical preparations.

Emulsifying agent

0.1–0.5

Gelling agent

0.5–2.0

Suspending agent

0.5–1.0

Tablet binder

5.0–10.0

Description

Carbomers are white-colored, ‘fluffy’, acidic, hygroscopic powders with a slight characteristic odor.

Pharmacopeial Specifications

Carbomer 940 (0.5 w/v) — 40 000–60 000(a)

Typical Properties

Acidity/alkalinity

pH = 2.7–3.5 for a 0.5% w/v aqueous dispersion;

pH = 2.5–3.0 for a 1% w/v aqueous dispersion.

Density (bulk)

1,.76–2.08 g/cm3

Density (tapped):

1.4 g/cm3

Glass transition temperature:

100–1058C

Melting point

Decomposition occurs within 30 minutes at 2608C.

Moisture content

Normal water content is up to 2% w/w. However, carbomers are hygroscopic and a typical equilibrium moisture content at 258C and 50% relative humidityis 8–10% w/w. The moisture content of a carbomer does not affect its thickening efficiency,

CHAPTER - XI

EXPERIMENTAL PROTOCOL

11.1 STANDARD CURVE FOR ETORICOXIB

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride in sufficient quantity of distilled water and the volume made up to 1000(I.P 2007).

100mg of Etoricoxib is accurately weighed and dissolved in a small quantity of methanol and made up to 100ml with the buffer phosphate buffered saline pH 7.4. From this primary solution 10ml is pipetted out and made up to 100ml with phosphate buffered saline pH 7.4. From this secondary solution aliquots are taken to produce 1, 2, 3, 4, 5, 6, 7, 8, 9, 10µg/ml.

The absorbance of the resulting solution is measured at 235nm in the UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan) using phosphate buffered saline pH 7.4 as blank. The standard curve is plotted by taking concentration in X-axis and Absorbance in Y-axis. (Sridhar *et al,* 2008).

11.2 PREFORMULATION STUDIES

11.2.1 *FT-IR studies*

The possibility of drug-excipients (cholesterol, nonionic surfactants) interactions are further investigated by FT-IR spectrum study. The FT-IR spectrum of pure drug and combination of drug with excipient are obtained by using Perkin FT-IR Spectrophotometer (Ismail Mouzam *et al,* 2011, Gurrapu.A *et al,* 2011). The scanning range is 450-4000 cm^{-1} and the resolution is 4cm^{-1}. Samples are prepared in KBr pellets

11.2.2 *Differential scanning calorimetry*

Differential Scanning Calorimetry is performed using Perkin Elmer STA 6000 Thermal Analyzer. The instrument is calibrated with indium standard. Accurately weighed (it varies from 3mg-25mg) samples are placed in an open type ceramic sample pans. Thermo grams are obtained by heating the sample at a constant heating rate of 8ºC/minute. A dry purge of Argon gas (60ml/min) is used for all runs. Samples heated from 37ºC-400ºC (Malay K Das *et al,* 2011, Indu Pal Kaur *et al,* 2011, Gurrapu .A *et al,* 2011).

11.3 FORMULATION OF ETORICOXIB NIOSOMES

Different ratios of surfactant and cholesterol are used to prepare niosomes with the concentration of the drug being the same.

The niosome formulations are prepared by thin film hydration technique. The weighed amount of cholesterol, non-ionic surfactant (cholesterol: non-ionic surfactant in micromoles) dissolved in 5ml of solvent mixture (Chloroform: Methanol 2:1 ratio). It is then transferred to a 100ml round bottom flask. A thin film is formed under reduced pressure in a rotary flash evaporator rotated at 100rpm at 55ºC.

The organic solvent is evaporated to form a dry film on the walls of the flask. An appropriate amount of Etoricoxib dissolved in methanol and add 10 ml phosphate buffered saline pH 7.4 and this is added slowly to the round bottom flask having thin film of surfactant and cholesterol and vortexed continuously for a period of 45 minutes at 55ºC, until a good dispersion of the mixture is obtained (Pratap S. Jadon *et al,* 2009, Vijay Prakash pandey *et al,* 2009, Malay K Das *et al,* 2011, Jong Soo Woo *et al,* 2009). The niosomal dispersion is collected and stored at 4ºC for maturation.

The empty niosomes also prepared by the same method without the drug for further evaluation.

11.3.1 ***Drug content***

The amount of drug in the formulation is determined after lysing the niosomes using 50% n- propanol.

Niosomes preparation equivalent to 200µg of Etoricoxib (0.1ml) is pipetted out in 100ml standard flask. To this sufficient quantity of 50% n- propanol is added and shaken well for the complete lysis of the vesicles. The volume is made up to 100 ml with the buffer phosphate buffered saline pH 7.4.

The absorbance is measured at 235nm in the UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan) using empty niosomes as blank.

The drug content is calculated from the standard curve, by using the following formula,

$$\text{Drug content} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times 100.$$

11.3.2 Entrapment Efficiency

Etoricoxib niosome preparations (1 ml) are centrifuged at 14,000 rpm for 120 minutes at 4ºC using a refrigerated centrifuge (Eppendorf, 5417R, Germany) in order to separate niosomes from unentrapped drug. The free drug concentration in supernatant layer after centrifugation is determined at 235 nm using UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). The percentage of drug entrapment in niosomes is calculated using the following formula. (Vijay Prakash Pandey *et al,* 2009, Maly K Das *et al,* 2011, Gyanendra Singh *et al,* 2010).

$$\% \text{ drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant})}{\text{Total drug}} \times 100.$$

11.3.3 ***Invitro release studies***

Invitro release pattern of niosomes suspension is carried out by dialysis bag (Himedia dialysis membrane (mw 12,000) method. The niosomal preparation of Etoricoxib is placed in a dialysis bag with an effective length of 5 cm which acts as a donor compartment. Dialysis bag is placed in a beaker containing 250 ml of buffer phosphate buffered saline pH 7.4, which acts as receptor compartment. The temperature of receptor medium maintained at 37±1ºC and the medium is agitated at 50 rpm speed using magnetic stirrer. Aliquots of 5 ml samples are collected at predetermined time and replenished immediately with the same volume of fresh buffer phosphate buffered saline pH 7.4 (Pavala Rani *et al,*2010, Ghada Abdelbary *et al,*2008, Ajay B.Solanki *et al,*2010, Florence A.T *et al,* 1994). The sink condition is maintained throughout the experiment. The collected samples are analyzed spectrophotometrically at 235 nm using UV-Visible Spectrophotometer (Shimadzu UV-1700 Pharma spec Japan). Each study is performed in triplicate (Prasun Bandyopadhyay *et al*, 2007, Manivannan Rangasamy *et al*, 2010).

The invitro release studies are also carried out for the pure drug by same method.

11.3.4 ***Invitro release kinetics***

In general drug release from vesicular system is controlled by various factors such as type of non-ionic surfactant, cholesterol content, membrane rigidity and lamellarity.

To understand the pharmacokinetics and mechanism of drug release, the result of *in-vitro* drug release study of niosomes were fit with various pharmacokinetic equations like zero order (cumulative % release vs time), first order (log % drug remaining vs. time), Higuchi's model

(cumulative % drug release vs. square root of time), and the Korsmeyer-Peppas (log cumulative % drug release vs log time) and Hixson-Crowel models (cubic root of drug remaining vs time). The r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots. (Ismail Mouzam *et al,* 2011, Gyanendra Singh *et al,* 2010,Abul Kalam Lutful Kabir *et al.,*2009, Harris Shoaib. M *et al,*2006).

The data are evaluated according to the following equation.

- Zero order - $Q = K_O t$
- First Order – Log Q = $LogQ_0$-kt / 2.303
- Higuchi – Qt = KH t½
- Korsmeyer-Peppas - M_t M=$K_t \infty$ / Kt_n
- Hixson – Crowell – Q 1/3 – Q_t 1/3 =

11.4 FORMULATION OF ETORICOXIB NIOSOMAL GEL

On the basis of entrapment efficiency and in-vitro release studies, maximum entrapment and sustained release niosomal dispersion is selected for the preparation of topical gel system.

Carbopol 934, (as a gelling agent in 0.9% w/w concentration) is dispersed in to distilled water. The dispersion is allowed to hydrate for 4-5 hours. Niosomes equivalent to 1% w/v of Etoricoxib is incorporated in to aqueous dispersion of polymers. The resultant dispersion after uniform mixing is neutralized and made viscous by the addition of tri ethanolamine (5% w/v) to obtain a translucent gel. (Ajay B.Solanki *et al,* 2010, Abdul Hasan Sathali *et al,* 2010). Niosomal gels are evaluated for Drug content, pH, Rheological behaviors, Particle size, Zeta potential, Transmission electron microscopy, *Invitro* release, *In vivo* studies, Stability studies and Statistical analysis.

11.5 EVALUATION OF THE PREPARED ETORICOXIB NIOSOMAL GEL

11.5.1 *Drug content*

Etoricoxib is extracted from 1gm of each gel formulations with methanol and diluted with phosphate buffer saline pH 7.4. The resultant mixture is filtered through membrane filter (pore size 0.45 mm). The absorbance of the sample solution is determined by using uv-spectrophotometer at 235 nm (Shimadzu UV-VIS spectrophotometer) after appropriate dilution with phosphate buffer saline (pH 7.4) (Patel R.P *et al,* 2009).

11.5.2 *pH measurements*

The pH of the gel formulations is determined using a pH meter. This is calibrated before each use with buffered solutions at pH 4, 7 and 10.Measurement is performed at1st, 15th and 30th day after preparation to detect any pH fluctuations (Abdul Hasan Sathali *et al,* 2011, Patel R.P *et al*, 2009).

11.5.3 *Rheology studies*

The viscosity of topical niosomal gel formulation is an important factor in determining residence time of drug in the skin. The viscosity determinations of prepared formulations are carried out using Brookfield DV-111+ Rheometer with spindle LV-3. The prepared system is allowed to gel in the STF and then viscosity is measured. The viscosity of samples is measured at different angular velocities. A typical run comprised changing angular velocity from 10 to 100 rpm with equal wait for each rpm. The hierarchy of angular velocity is reversed (100 to 10 rpm) with similar wait. The averages of two readings are used to calculate the viscosities of formulations (Kapadia *et al,* 2009, Abdul Hasan Sathali *et al,* 2010).

11.5.4 ***Particle size***

The vesicle sizes of prepared niosomal formulation composed of Span 60 and Tween 80 and cholesterol in a 6:1 and 3:1 micro molar ratio were determined by light scattering based on laser diffraction using the Malvern master-sizer (Malvern Instrument Ltd, Worcestershire, UK). All measurement was conducted at 25ºC (Arunothayam *et al,* 2000, Zidan.A.S *et al,* 2011, Xu.X *et al,* 2011).

11.5.5 ***Zeta potential***

Zeta potential is measured by using a Malvern ZS 90 zeta-sizer and a folded capillary cell. The tests are conducted at 25ºC (Xu.X *et al*, 2011). The time dependent correlation function on the scattered light intensity is measured at a scattering angle of 90ºC (Manosroi.A *et al*, 2010, Phikunthong Kopermsub *et al,* 2011, Ahmed S. Zidan *et al,* 2011).

11.5.6 Transmission electron microscopy

The prepared niosomal gel formulation is characterized for their morphology using transmission electron microscopy (TEM). Briefly, to an small amount of prepared niosomal gel formulation, sufficient quantity of 1% phosphotungstic acid is added and mixed gently. A small amount of the gel is placed on the carbon coated grid and drained off the excess. The grid was allowed to dry and it is observed under transmission electron microscopy. (Hitachi model H-T 100, Japan) (Pratap S. Jadon *et al,* 2009, Ammar.O.A *et al,* 2011, Varaporn Buraphacheep Junyaprasert *et al,* 2008).

11.5.7 ***Invitro release studies***

In-vitro release study is carried out by taking 1 g of gel formulations into dialysis bag and placed beaker containing 250 ml PBS pH 7.4 at 37 ± 10˚C. The beaker is placed over a magnetic stirrer and stirred at constant speed. Aliquots of samples are withdrawn at specified time

intervals and analyzed at 235 nm by using an UV spectrophotometer to determine the percentage drug released and replaced with equal volume of fresh PBS pH7.4 (Ajay B Solanki *et al,* 2010).

11.5.8 Invitro release kinetics

To understand the pharmacokinetics and mechanism of drug release, the result of *in-vitro* drug release study of niosomes were fit with various pharmacokinetic equations like zero order (cumulative % release vs time), first order (log % drug remaining vs. time), Higuchi's model (cumulative % drug release vs. square root of time), and the Korsmeyer-Peppas (log cumulative % drug release vs log time) and Hixson-Crowel models (cubic root of drug remaining vs time). The r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.

The data are evaluated according to the following equation.

- Zero order - $Q = K_O t$
- First Order – $\text{Log } Q = \text{Log} Q_0 - kt / 2.303$
- Higuchi – $Qt = KH\ t^{1/2}$
- Korsmeyer-Peppas - $M_t\ M = K_t \infty / Kt_n$
- Hixson – Crowell – $Q\ 1/3 – Q_t\ 1/3$

11.5.9 In vivo anti-inflammatory studies

The *in-vitro* and *in vivo* studies are carried out and all the study protocols are approved by the local institutional Animal Ethical Committee. The anti-inflammatory activity is compared by the carrageenam induced rat paw edema method. The studies are conducted on albino rats of either sex, weighing 200 ± 20 g. The animals in each group (n = 3) are selected so that the average body weight among the groups is as close as possible. Inflammation is produced in the rats by injecting 0.1 ml of 1% w/v carrageenam suspension in saline, into the sub-plantar surface

of the rats' left hind paw. Thirty minutes later, 0.5 g niosomal gel formulations and plain gel (Drug dispersion in polymers) are applied topically on the edematous paw by gently rubbing with an index finger. Topical activity of the various formulations is evaluated by measuring an increase in the hind paw thickness with the help of digital calipers before (time 0) and 1, 2, 3, 4, 5, 6, and 24 h after carrageenam administration. The percentage of paw thickness increase from time 0 is calculated and compared with control group (Srikanth.K *et al,* 2010, Ajay B. Solanki *et al,* 2010, Ambikanandan Misra *et al,* 2002, Alok Namdeo *et al,*1999).

GROUP-1(STANDARD GROUP)

Animal were treated with carrageenan

GROUP-2(STANDARD GROUP)

Animals were treated with Etoricoxib plain gel

GROUP-3(TEST GROUP)

Animals were treated with Etoricoxib niosomal gel (High entrapment).

GROUP-4(TEST GROUP)

Animals were treated with Etoricoxib niosomal gel (Low entrapment).

The inhibition percentage of the edema formulation of the test samples were calculated and statistically evaluated ($p<0.05$) according to the following equation

(Manosroi .A *et al,* 2008, Eros.I *et al,* 2005).

Percentage of edema inhibition=Tc-Tt/Tc×100

Where

T_c is the mean edema thickness of rat paw edema control group

T_t is the mean edema thickness of rat paw in the test sample.

11.5.10 Stability studies

The best formulation of etoricoxib loaded niosomal gel is subjected to stability studies. The formulation is stored in two different temperatures, 4^0C (Refrigerator), 25 ± 2^0C/60% RH±5% RH in an environmental chamber [In lab equipments (Madras) Pvt. Ltd] for the period of 3 months (Indu Pal Kaur *et al,*2011, Raju Jukanti *et al,*2011, Abdul Hasan Sathali *et al,*2010, Rathi Jagdish Chandra *et al,* 2009). The drug content of the formulation is estimated every months

Analysis of variance

The data are expressed as the mean ± standard deviation are subjected to student t-test and one way analysis of variance (ANOVA) and the significance of difference between formulation is calculated by student- newman-keuls (compare all pairs) with (Insat Grap pad prism soft ware). The level of statistical significance is chosen as less than $p<0.05$ (Gurrapu *et al,* 2011, Shyh Darli *et al,* 2011).

CHAPTER- XII

RESULTS AND DISCUSSION

12.1 STANDARD CURVE FOR ETORICOXIB

The λ_{max} of Etoricoxib was determined by scanning the 10µg/ml of drug solution in phosphate buffer saline (PBS) pH7.4 and it showed the λ_{max} at 235nm. The λ_{max} of etoricoxib is showed as UV graph in Figure 13

Calibration curve of Etoricoxib was plotted by measuring the absorbance of different concentrations of the drug in phosphate buffer saline (PBS) pH 7.4 at 235 nm. The linear correlation co-efficient was obtained for calibration of Etoricoxib in phosphate buffer saline (PBS) pH7.4. (K.Srithar *et al,* 2005). Etoricoxib obeys the beer's law within the concentration range of 1 to 10µg/ml. Calibration readings were shown in Table III and the calibration plot of etoricoxib were shown in Figure14

12.2 PREFORMULATION STUDIES

12.2.1 *FT-IR studies*

FT-IR infra red (FT – IR) spectroscopy was carried out separately to check the compatibility between drug, surfactant (Span 20,Span 40,Span 60,Span 80, Tween 60,Tween 80 and Brij-52) cholesterol and physical mixture used for the preparation of niosomes.

The spectra studied at 4000cm^{-1} to 400 cm^{-1} were shown in Figure 15 and Table IV. It was found from the spectra that there was no major shifting as well as any loss of functional peaks in the spectra of drug, surfactant, cholesterol and physical mixture of drug, surfactants and cholesterol The results indicated that the selected surfactants and cholesterol were found to be

compatible with the selected drug (Gurrapu *et al,* 2011, Ismail Mouzam *et al,* 2011, Pandey Shivanand *et al,* 2010).

12.2.2 *Differential scanning calorimetry*

DSC is a fast and reliable method to screen drug- excipients interactions as indicated by appearance of a new peak, change in the peak shape and its onset, peak temperature/melting point and relative peak area or enthalpy Figure 16 depicts various DSC thermograms such as pure drug, span20, span40, span60, span80, tween60, brij52 and cholesterol obtained during the study. Pure etoricoxib showed a sharp endothermic peak at 153º.09ºC.Thermogram of span20,span40,span60,tween60,brij52 exhibits an endothermic peak with onset at 160º.51ºC, 48ºC, 58ºC, 6.33ºC, 172ºC respectively. Tween 80 is reported to show an exothermal peak at -43ºC and endothermal peaks were observed at -14ºC (Hillgren *et al,* 2002, Indu Pal Kaur *et al,* 2011).

Further, DSC thermogram suggests that the formulation components Span 20, Span 40 Span 60, Tween 60, brij-52, cholesterol and the drug etoricoxib do not interact to form any additional chemical entity but remain as mixture.

12.3 FORMULATION OF ETORICOXIB NIOSOMES

Niosome formulations were formulated by thin film hydration method using different surfactants (Span 20, Span 40, Span 60, Span 80, Tween 60, Tween 80, and Brij 52) and Cholesterol with different ratios as per the formula given in Table V resulted a stable, uniform dispersion of niosomal vesicles (Pratap S. Jadon *et al,* 2009, Vijay Prakash pandey *et al*, 2009, Malay K Das *et al*, 2011, Jong Soo Woo *et al*, 2009). The formation of niosomal vesicle was confirmed by Transmission electron microscopy (TEM)

12.3.1 *Drug content*

The drug content of the niosomes was determined after lysing the vesicles with 50% n-propanol and measured at 235 nm in UV-Visible spectrophotometer 1700, Pharma spec, Japan. The drug content was found to be in the range of 97.23% to 99.46%. The results were indicated that the uniform distribution of drug in prepared niosomal formulations. The observed results were shown in Table VI.

12.3.2 *Entrapment efficiency*

In niosomal formulations, the impact of surfactant and cholesterol concentration on entrapment efficiency was considerably significant. The ranges of entrapment efficiency of twenty eight niosomal formulations were observed about 71.6% to 96.9% and are shown in Table VI and Figure 17. The highest entrapment efficiency obtained for the formulation prepared with F12 Span60 (6:1) (300µmol surfactant and 50µmol cholesterol) was found to be 96.9% may be due to surfactant chemical structure (Span series) and having highest phase transition temperature.

Effect of surfactant on entrapment efficiency

Formulations F1-F4 were prepared with different ratios of surfactant (Span 20) and cholesterol(3:1,4:1,5:1 and 6:1) showed the entrapment efficiency of 82.7%, 84.9%, 85.8% and 87.3% respectively.

Formulations F5-F8 were prepared with different ratios of surfactant(Span 40) and cholesterol(3:1,4:1,5:1 and 6:1) showed the entrapment efficiency of 86.5%, 87.9%, 88.1% and 89.2% respectively.

Formulations F9-F12 were prepared with different ratios of surfactant (Span 60) and cholesterol(3:1,4:1,5:1 and 6:1) showed the entrapment efficiency of 94.6%,95.8%,96.3% and 96.9% respectively.

Formulations F13- F16 were prepared with different ratios of surfactant (Span 80) and cholesterol(3:1,4:1,5:1 and 6:1) showed the entrapment efficiency of 77.5%,78.2%,80.8% and 81.6 % respectively.

Formulations F17- F20 were prepared with different ratios of surfactant (Tween 60) and cholesterol (3:1, 4:1, 5:1 and 6:1) showed the entrapment efficiency of 75.6%, 77.6%, 78.7% and 80.9 % respectively.

Formulations F21- F24 were prepared with different ratios of surfactant (Tween 80) and cholesterol (3:1, 4:1, 5:1 and 6:1) showed the entrapment efficiency of 71.4%, 72.9%, 74.1% and 76.6 % respectively.

Formulations F25- F28 were prepared with different ratios of surfactant (Brij-52) and cholesterol (3:1,4:1,5:1 and 6:1) showed the entrapment efficiency of 83.6%,85.9%,87.7% and 89.9 % respectively.

The entrapment efficiency of various nonionic surfactants increases in the order of Span 60 > Span 40 >Span 20 > Brij 52 > Span 80>Tween60 >Tween 80.

These results explained that the Span 60 has higher entrapment efficiency than other Span types and Tween 60, Tween 80 and Brij 52. This can be explained by many facts: a) Span 60 has the highest phase transition temperature (Yoshioka et al., 1994). (b) The length of alkyl chain of surfactant is a crucial factor in permeability. Long Chain surfactant produces high entrapment (Hao et al., 2002). Span 60 has a longer saturated alkyl chain (C16) compared to Span40 and Span20, so it produces niosomes with higher entrapment efficiency but Span80 has

unsaturated alkyl chain (C18) produces less entrapment. c) The longer alkyl chain influences the HLB value of the surfactant mixture which by its turn directly influences the drug entrapment efficiency (Raja Naresh et al., 1994). The lower the HLB of the surfactant the higher will be drug entrapment efficiency. (Guinedi.A.S *et al,* 2005).

In addition, entrapment efficiency for niosomes prepared with Tween 60 was higher than that of Tween 80 .This shows that the longer the alkyl chain of the surfactant, the less drug will be entrapped. Tween 80 has a longer saturated alkyl chain than tween 60 and lower entrapment efficiency. The length of the alkyl chain influences the hydrophilic- lipophilic balance (HLB) value of the surfactant, the lower will be the entrapment efficiency (Ruckmani *et al,* 2009, Ghada Abdelbary *et al,* 2008, Srikanth.K *et al,* 2010).

12.3.3 ***In vitro Release Studies***

The *in vitro* drug release studies of etoricoxib from niosomes were carried out by dialysis bag diffusion technique in phosphate buffer saline of pH7.4.

The cumulative % drug release at 12 hours was 92.9% for formulation F1 whereas it was 90 %, 86.4 % and 83.5 %, for formulations F2, F3 and F4, respectively.

The cumulative % drug release at 12 hours was 85.3% for formulation F5 whereas it was 83.6 %, 77.6 %and 74.2 %, for formulations F6, F7 and F8, respectively.

The cumulative % drug release at 12 hours was 78.5% for formulation F9 whereas it was 69.8 %, 62.2 % and 55.3 % for formulations F10, F11 and F12, respectively.

The cumulative % drug release at 12 hours was 96.1% for formulation F13 whereas it was 89.9 %, 87.3 % and 86.0 % for formulations F14, F15 and F16, respectively.

The cumulative % drug release at 12 hours was 94.0% for formulation F17 where as it was 87.5%, 82.7% and 76.4% for formulations F18, F19, and F20 respectively.

The cumulative % drug release at 12 hours was 96.05% for formulation F21 where as it was 90.4%, 83.6% and 76.1% for formulations F22, F23 and F24 respectively.

The cumulative % drug release at 12 hours was 81.3% for formulation F25 where as it was 75.3%, 74.1% and 66.7% for formulations F26, F27 and F28 respectively.

Significant changes in release were observed upon changing the type of surfactant used in the bilayer of etoricoxib niosomes. The results were shown in Table VII a, b, c, d, e, f, g and Figure 18 a, b, c, d, e, f, g.

The experimental studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. This result was in conformity with the report of (Anand Kumar.A *et al,* 2010, Samar Mansour *et al,* 2005, Arora Rajnish *et al,* 2010, Ghada Abdelbary *et al,* 2008).

All niosome formulations showed significant slower release than etoricoxib solution (10mg/0.5ml) which showed a release of about 96.6 % within 7 hours. This confirmed that a sink condition for etoricoxib release was accomplished and the dialysis bag used in the dissolution procedure did not limit etoricoxib release. Niosomal etoricoxib formulations with Span 60, Span 40, Brij52, Span 20 Span 80, Tween60 and Tween80 showed significant reduction in *in vitro* drug release in 12hours compared with pure drug in solution.

Effect of surfactants on the release rate of etoricoxib from niosomes:

From the release studies F12 (span 60 6:1) showed slower and prolonged drug release than the other formulations. This can be explained by higher entrapment efficiency (Ruckmani *et al,* 2009) and the fact that niosomes exhibit an alkyl chain length-dependent release (Guinedi. A.S *et al*, 2005). Further the release studies of the prepared formulations containing Span 20, 40, 80, Tween60, Tween80 and Brij52 were compared.

Among the four formulations from F1 to F4 prepared from span 20 at different ratios (3:1, 4:1, 5:1, and 6:4) F1 shows maximum drug release in 12 hours. The order of decreasing percentage drug release in 12^{th} hour were F1 > F2> F3 > F4.

Among the four formulations from F5 to F8 prepared from span 40 at different ratios (3:1, 4:1, 5:1, and 6:4) F5 shows maximum drug release in 12 hours. The order of decreasing percentage drug release in 12^{th} hour were F5 > F6> F7 > F8.

Among the four formulations from F9 to F12 prepared from span 60 at different ratios (3:1, 4:1, 5:1, and 6:1) F9 shows maximum drug release in 12 hours. The order of decreasing percentage drug release in 12^{th} hour were F9 > F10> F11 > F12.

Among the four formulations from F13 to F16 prepared from span 80 at different ratios (3:1, 4:1, 5:1, and 6:1) F13 shows maximum drug release in 12 hours. The order of decreasing percentage drug release in 12^{th} hour were F13 > F14> F15 > F16.

Among the four formulations from F17 to F20 prepared from Tween60 at different ratios (3:1, 4:1, 5:1, and 6:1) F17 shows maximum drug release in 12 hours. The order of decreasing percentage drug release in 12^{th} hour were F17 > F18> F19 > F20.

Among the four formulations from F21 to F24 prepared from Tween80 at different ratios (3:1, 4:1, 5:1, and 6:1) F17 shows maximum drug release in 12 hours. The order of decreasing percentage drug release in 12^{th} hour were F21 > F22> F23 > F24.

Among the four formulations from F25 to F28 prepared from Brij52 at different ratios (3:1, 4:1, 5:1, and 6:1) F25 shows maximum drug release in 12 hours. The order of decreasing percentage drug release in 12^{th} hour were F25 > F26> F27 > F28.

The entire amount of loaded drug was not released from the niosomes. This may be due to entrapment of the drug in the lipophilic region (Uchebhu.I.F *et al*, 1998).

The comparative release data indicate that, by encapsulation of drug into niosomes, it is possible to sustain and control the release of drug for longer duration (Ruckmani *et al,* 2000, Samar Mansour *et al,* 2005).

12.3.4 ***Invitro release kinetics***

The release constant was calculated from the slope of the appropriate plots and the regression coefficient (r^2) were extrapolated by zero order, first order, Higuchi, Korsmeyer-peppas and Hixson- Crowell equations to know the mechanism of drug release from these formulations (F1 to F28). In this study , the invitro release profiles of drug from the all formulations could be best expressed by zero order and Higuchi equation, as the plots showed highest linearity (r^2=0.977 to =0.998) and (r^2=0.896 to=0.978).To confirm the diffusion mechanism, the data were fitted into Korsmeyer-peppas equation. All the formulations showed good linearity (r^2=0.954 to 0.994), with slope (n) values ranging from 0.693 to 0.1.061 indicating that diffusion was the predominant mechanism of drug release from these formulations. The release profile of etoricoxib from all these formulations displayed very poor fitting with Hixson Crowell cube root model drug release ((Kabir *et al,* 2009, Almira *et al,* 2001). The results were shown in Figure 19 a, b, c, d, e, f, g and Table VIII a, b, c, d, e, f, g.

Among the all formulations was found, that the invitro release of F12 – Span60 (6:1) was best explained by higuchi equation, as the plots showed the highest linearity (r^2 =0.978), followed by zero order (r^2 =0.989) and first order (r^2 =0.958). The Korsmeyer-peppas equation indicated good linearity (r^2 =0.996).The release exponent n was 0.778 which appears to indicate a coupling of the diffusion and erosion mechanism-so-called anomalous diffusion and may indicate that the drug release is controlled by more than one process. Thus F12 was selected as the best for niosomal release of etoricoxib (Gyanendra Singh *et al,* 2010).

These results pointed to sustained release characteristics with a higuchi pattern of the drug release, where niosomes act as reservoir system for continuous delivery of drug. This slow release pattern of entrapped drug may indicate the stability of the niosomal formulations (Ismail. A. Attia *et al,* 2007).

12.4 FORMULATION OF ETORICOXIB NIOSOMAL GEL

The selected best niosomal formulation (on the basis of highest (F12Span 60, - 6:1) and lowest (F21 Tween 80, 3:1) entrapment efficiency among the all formulations) was incorporated into suitable gel base (Carbopol 940 as gelling agent 0.9%) to obtain 1% of the drug and plain etoricoxib gel was prepared by incorporating the drug into suitable gel base to obtain same 1% of the drug. Both the formulas were showed in Table IX a and IX b.

12.5 EVALUATION OF THE PREPARED ETORICOXIB NIOSOMAL GEL

12.5.1 *Drug Content*

The drug content of the prepared gels was determined after lysing the vesicles with 50% n- propanol. It was measured at 235 nm at UV-visible spectrophotometer, 1700-pharma spec, Japan. The drug content was to be 98.7% for FG12 (span60 6:1) and 99.02% for FG21 (Tween 80 3:1) respectively. This indicated that the uniform distribution of drug in prepared gel formulations. The results were in shown Table X.

12.5.2 *pH measurement*

The result of pH measurement showed that all niosomal gel formulations which were prepared have the pH range in between 6.9 to 7.0 at intervals of 0 , 15 ,30 th day respectively (Patel. R.P *et al,* 2009). This is considered acceptable to avoid the risk of irritation upon application to the skin (Japan Patel *et al,* 2011). The results were shown in Table XI.

12.5.3 *Rheological Studies*

The viscosity of the selected formulations based on entrapment efficiency and in vitro release. The viscosity of all these formulations decreased as the shear rate increased, which showed the character of pseudoplastic fluid.

Formulation FG-12 showed better pseudoplastic behavior compared to FG-21 formulations. The formulation FG-12 selected as best formulation compared to that of FG-21 formulations based on clarity, pH, *in vitro* release and viscosity. The pseudoplastic behaviors of formulations were showed in Table XII. (Abdul Hasan Sathali *et al,* 2010, Kapadia *et al,* 2009)

12.5.4 Particle *size*

The mean particle diameters of topical niosomal gel composed of Span 60 and cholesterol in a 6:1(FG12) micro molar ratio were 1368nm, while the mean particle diameters of topical niosomal gel composed of Tween 80 and cholesterol in a 3:1(FG21) micro molar ratio were 1206nm. The results reveal that the niosomes prepared using Span 60 was larger in size than niosomes prepared using Tween80 due to Span 60 has a longer saturated alkyl chain compared to Tween 80 having unsaturated alkyl chain. And it was reported that surfactants with longer alkyl chains generally gave larger vesicles. This would account for the higher entrapment efficiencies obtained with Span 60 topical niosomal gel. This suggests that when the hydrophobicity of the surfactant increases, the vesicle size increases. Similar results were observed in (Agarwal *et al,* 2001, Manosroi.A *et al,* 2003, Guinedi A.S *et al,* 2005, Sankar *et al,* 2009).The results were shown Figure 20.

12.5.5 *Zeta potential studies*

The zeta potential was used to study the surface charge analysis of formulation (FG 12) Surfactant and cholesterol in a 6:1 micro molar ratio were found to be-76mV and formulation (FG 21) Surfactant and cholesterol in a 3:1 micro molar ratio were found to be-89mV respectively. (Vijay Prakash Pandey *et al.,* 2009). Zeta potential values increased with the hydrophilicity of the surfactants increased. This could be due to the fact that the surface free energy of the span surfactants increases with increased HLB value (Uchegbu *et al,* 1995). On the other hand, increasing the hydrophilicity of the surfactant was associated with a decrease of the niosomal size (Zidan A.S *et al,* 2011).

12.5.6 *Transmission electron microscopy*

Transmission electron microscopy was performed to study vesicle morphology that revealed that niosomes gel were discrete, and had spherical in shape as showed in Figure 21a &21b (Pratap S. Jadon *et al,* 2009, Ammar.O.H *et al,* 2011, Ismail Mouzam *et al,* 2011).

12.5.7 *Invitro* release studies

The *in vitro* drug release study of etoricoxib niosomal gel was carried out by dialysis bag diffusion technique in phosphate buffer saline of pH7.4.

The cumulative % drug release at 12 hours was 58.8% for etoricoxib niosomal gel containing (GF12- Span 60 (6:1) , highest entrapment 96.9%) whereas it was 95.1 %, for etoricoxib niosomal gel containing (GF21- Tween 80 3:1 lowest entrapment 71.46%) respectively. The results were shown in Table XIII and Figure 22.

The cumulative % drug release at 7 hours was 98.8% for plain etoricoxib gel, when compared to niosomal gel GF-12 (58.8%) and GF-21 (95.1%) respectively.

From the results, it was concluded that (GF-12) etoricoxib niosomal gel showed prolong drug release due to the highest entrapment efficiency when compared to that of F-21 and plain etoricoxib gel (Aliasgar Shahiwala *et al,* 2002, Malay K. Das *et al,* 2010, Mahmoud Mokhtar *et al,* 2008, Ajay B. Solanki *et al,* 2010).

12.5.8 *Invitro* release kinetics

The amount of drug release from different etoricoxib niosomal gel formulations (GF12 and GF21) shows a linear relationship with square root of time. Hence, the drug release rate can be expressed by higuchi diffusion model (r^2=0.979 to 0.944). The high correlation coefficients were obtained for the zero order drug release kinetics for etoricoxib niosomal gel was found to be (r^2=0.990 to 0.991). The results were shown in Table XIV and Figure23. (Ajay B. Solanki *et al,* 2010).

The n value obtained from Korsmeyer – peppas equation found to be 0.634 to 1.309 which indicate that the formulation GF12 and GF21 showed drug release by Non-Fickian and case 2 transport diffusion mechanisms. (Abdul Hasan Sathali *et al,* 2011).

12.5.9 *Invivo-anti inflammatory studies*

In vivo anti-inflammatory study was used to evaluate the therapeutic efficacy of a niosomal gel formulation. The study was carried out by albino rat using rat paw edema method. Niosomal topical gel formulation FG 12 (span60 6:1)23.9% showed sustained reduction in the paw thickness for all points of time when compared to that of FG21 (Tween 80 3:1)46.0% and 59.6% for plain gel. The results were shown in Table XV and Figure 24. Significant differences in the decrease in paw thickness among all formulations showed ($p<0.05$). This result suggested that prolonged reduction in the paw thickness due to high drug entrapment and controlled invitro drug release (Ajay B. Solanki *et al,* 2010, Eros I *et al,* 2005, Abdul Hasan Sathali *et al,* 2010).

12.5.10 ***Stability studies***

The percentage drug content of was monitored for etoricoxib niosomal gel formulation (FG12) upon storage at refrigerated temperature $4^{o}\pm2^{o}C$ and $25^{o}C\pm60\%$ RH at accelerated stability chamber for a period of 3 months (Abdul Hasan Sathali *et al,* 2011).

The stability studies suggest that the etoricoxib niosomal gel formulations were comparatively more stable at refrigerated conditions compared to accelerated temperature. The results were shown in Table XV a and XV b (Gurrapu.A *et al.,* 2011).

TABLE III CALIBRATION OF ETORICOXIB AT 235nm (PBS OF pH 7.4)

S.NO	CONC(µg/ml)	ABSORBANCE AT 235nm (PBS PH 7.4)			AVERAGE AND STANDARD DEVIATION
		TRIAL-1	TRIAL-2	TRIAL-2	
1	1	0.073	0.078	0.082	0.077±0.004
2	2	0.158	0.144	0.166	0.156±0.009
3	3	0.234	0.236	0.233	0.234±0.001
4	4	0.293	0.290	0.292	0.291±0.001
5	5	O.367	0.368	0.370	0.368±0.001
6	6	0.437	0.438	0.440	0.438±0.001
7	7	0.498	0.502	0.504	0.501±0.001
8	8	0.562	0.586	0.581	O.576±0.010
9	9	0.645	0.650	0.657	0.650±0.004
10	10	0.718	0.717	0.721	0.718±0.001

n=3*

TABLE IV IR PEAKS OF DRUGS, SURFACTANTS, CHOLESTEROL AND PHYSICAL MIXTURE OF DRUG, SURFACTANTS AND CHOLESTEROL

S. No	Description	Characteristic peaks (cm^{-1}) obtained
1	Etoricoxib	3753.57 , 3438.85, 3057.58 , 2923.70 , 2855.50,2368.48, 1599.25, 1497.80 , 1431.88 1403.78 ,1299.11, 1144.12 , 1085.54 ,1014.26 959.44,840.10, 777.91 , 736.14, 636.37 , 582.83,543.67, 492.83, 464.80
2	Cholesterol	3402.2,2933.53,2900.74,2867.95,1670.24, 1620.09, 1465.8, 1440.73, 1375. 15,1274.86, 1236.29, 1191.29, 1164.92, 1134.07, 1107.06, 1054.99,1022.2,985.56,956.63,927.7,883.34, 838.98, 800.4,736.76,597.89,501.41.
3	Span 20	3396.41,2923.88,2852.52,1741.6,1461.94,1375.15,1172.64, 1110.92, 1076.21,981.7,923.84,881.41,838.98,773.4,723.26,609. 46.372.24.
4	Span 40	3379.05,2918.1,2850.59,1735.81,1465.8,1382.87,1288.36, 1267.74,1245.93,1224.71,1176.5,1091.63, 1056.92, 979.77,883.34, 811.98, 777.26,721.36.
5	Span 60	3407.98,2918.1,2850.59,1735.81,1467.65,1380.94, 1265.22, 1244, 1220.86, 1176.5,1097.5,1056.92, 885.27,721.33.
6	Span 80	3396.41,2923.88,2854.45,1739.67,1652.88,1461.94,1415.65, 1377.08,1238.21,1174.57,1110.92,881.41,723.26, 609.26, 376.09.
7	Physical mixture of Drug, Span 20 and Cholesterol	3754.33, 3400.44, 2926.88 , 2857.09, 2372.15, 1741.85 , 1655.67, 1023.89 , 458.52
8	Physical mixture of Drug, Span 40 and Cholesterol	3753.76, 3431.95, 2927.29, 2368.35 , 1599.38, 1432.03, 1298.98, 1144.00, 1020.32, 839.83, 777.96, 582.93, 543.93, 464.19 ,400
9	Physical mixture of Drug, Span 60 and Cholesterol	3753.84, 3422.05, 2925.65, 2369.42, 1741.78 , 1599.75 , 1431.83, 1299.50, 1144.57, 1056.22, 839.51, 777.49, 544.10 ,
10	Physical mixture of Drug, Span 80 and Cholesterol	3753.76, 3401.13, 2926.33, 2372.17, 1655.31, 1459.12, 1023.93, 456.17,400
11	Physical mixture of Drug, Tween 60 and Cholesterol	3404.46, 2924.91, 2367.85, 1738.42, 1655.09, 1459.65, 1108.39, 400
12	Physical mixture of Drug, Tween 80 and Cholesterol	3753.91, 3402.90, 2926.93, 2369.10, 1737.08, 1655.66, 1459.43, 1351.58, 1107.58,400

TABLE V FORMULATION OF ETORICOXIB NIOSOMES

S.NO	FORMULATION	SURFACTANT	RATIO	
			SURFACTANT	CHOLESTEROL
1	F1	SPAN 20	3	1
2	F2	SPAN 20	4	1
3	F3	SPAN 20	5	1
4	F4	SPAN 20	6	1
5	F5	SPAN 40	3	1
6	F6	SPAN 40	4	1
7	F7	SPAN 40	5	1
8	F8	SPAN 40	6	1
9	F9	SPAN 60	3	1
10	F10	SPAN 60	4	1
11	F11	SPAN 60	5	1
12	F12	SPAN 60	6	1
13	F13	SPAN 80	3	1
14	F14	SPAN 80	4	1
15	F15	SPAN 80	5	1
16	F16	SPAN 80	6	1
17	F17	TWEEN 60	3	1
18	F18	TWEEN 60	4	1
19	F19	TWEEN 60	5	1
20	F20	TWEEN 60	6	1
21	F21	TWEEN 80	3	1
22	F22	TWEEN 80	4	1
23	F23	TWEEN 80	5	1
24	F24	TWEEN 80	6	1
25	F25	BRIJ-52	3	1
26	F26	BRIJ-52	4	1
27	F27	BRIJ-52	5	1
28	F28	BRIJ-52	6	1

Drug concentration used in each formulation kept as constant 200mg/10ml.

n=3*

TABLEVI % ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS

S.NO	FORMULATION	SURFACTANT	RATIO		% DRUG CONTENT	% ENTRAPMENT
			SURFACTANT	CHOLESTEROL		
1	F1	SPAN 20	3	1	97.54	82.7±0.98
2	F2	SPAN 20	4	1	99.43	84.9±0.87
3	F3	SPAN 20	5	1	98.41	85.8±0.12
4	F4	SPAN 20	6	1	97.86	87.3±0.76.
5	F5	SPAN 40	3	1	97.65	86.5±0.87
6	F6	SPAN 40	4	1	98.76	87.9±0.65
7	F7	SPAN 40	5	1	98.97	88.1±0.88
8	F8	SPAN 40	6	1	99.09	89.2±0.98
9	F9	SPAN 60	3	1	99.34	94.6±0.76
10	F10	SPAN 60	4	1	97.54	95.8±1.09
11	F11	SPAN 60	5	1	97.86	96.3±0.98
12	F12	SPAN 60	6	1	99.12	96.9±0.76
13	F13	SPAN 80	3	1	99.43	77.5±0.56
14	F14	SPAN 80	4	1	97.56	78.2±0.77
15	F15	SPAN 80	5	1	98.33	80.8±0.68
16	F16	SPAN 80	6	1	98.57	81.4±0.99
17	F17	TWEEN 60	3	1	99.07	75.6±0.56
18	F18	TWEEN 60	4	1	99.32	77.6±0.99
19	F19	TWEEN 60	5	1	98.45	78.7±0.68
20	F20	TWEEN 60	6	1	98.77	80.9±0.95
21	F21	TWEEN 80	3	1	97.98	71.4±0.56
22	F22	TWEEN 80	4	1	99.70	72.9±0.54
23	F23	TWEEN 80	5	1	99.56	74.1±0.44
24	F24	TWEEN 80	6	1	98.65	76.6±0.76
25	F25	BRIJ-52	3	1	98.79	83.6±0.87
26	F26	BRIJ-52	4	1	99.43	85.9±0.88
27	F27	BRIJ-52	5	1	99.54	87.7±0.99
28	F28	BRIJ-52	6	1	98.37	89.8±0.77

n=3*

TABLE VIIa *invitro* CUMULATIVE % DRUG RELEASE PROFILE OF NIOSOME CONTAINING SPAN-20 IN DIFFERENT RATIO

***invitro* RELEASE MEDIUM**	**TIME IN HOURS**	**BATCH CODE(SURFACTANT:CHOLESTEROL RATIO)**			
		F1(3:1)	**F2(4:1)**	**F3(5:1)**	**F4(6:1)**
		MEAN±SD	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
PBS pH 7.4	0.25	6.0±0.26	3.5±0.41	2.4±0.24	1.8±0.44
	0.50	8.5±0.28	6.5±0.28	5.1±0.46	3.7±0.45
	0.75	10.8±0.36	7.5±0.29	6.9±0.40	5.7±0.32
	1	12.8±0.33	11.7±0.16	9.2±0.36	8.1±0.45
	1.5	16.0±0.49	14.4±0.32	11.2±0.32	10.2±0.28
	2	18.8±0.49	17.3±0.36	13.7±0.36	12.3±0.53
	2.5	22.4±0.40	19.7±0.30	16.3±0.41	14.4±0.40
	3	25.1±0.48	21.9±0.40	18.7±0.41	16.7±0.38
	3.5	28.8±0.40	24.7±0.37	21.5±0.40	19.2±0.24
	4	31.8±0.35	27.4±0.24	24.5±0.49	20.9±0.67
	4.5	34.9±0.35	30.7±0.28	28.4±0.53	23.0±0.53
	5	38.3±0.44	33.2±0.45	31.5±0.73	26.3±0.57
	5.5	41.8±0.45	36.4±0.32	35.2±0.69	29±0.49
	6	45.6±0.52	41.8±0.21	39.6±0.37	33.6±0.28
	6.5	48.9±0.48	44.7±0.37	42.9±0.37	37.3±0.45
	7	52.5±0.41	49.0±0.29	46.4±0.37	40±0.45
	7.5	56.5±0.53	53.0±0.29	49.2±0.58	43.5±1.11
	8	60.3±0.44	56.4±0.29	52.1±0.38	46.9±1.75
	8.5	64.6±0.49	59.7±0.43	55.3±0.57	52.8±1.46
	9	68.8±0.32	63.2±0.28	58.1±0.41	56.4±0.58
	9.5	72.8±0.67	67.2±0.37	61.1±0.21	60.5±0.80
	10	76.9±0.79	70.8±0.37	65.9±0.63	63.9±0.49
	10.5	80.9±0.57	74.7±0.32	71.3±0.49	68.1±0.36
	11	84.8±0.77	79.3±0.49	75.2±0.69	72.9±0.61
	11.5	88.6±0.61	84.3±0.38	80.9±0.41	78.3±0.32
	12	92.9±1.17	90±0.58	86.4±0.62	83.5±1.02

n=3*

TABLE VIIb *invitro* CUMULATIVE % DRUG RELEASE PROFILE OF NIOSOME CONTAINING SPAN-40 IN DIFFERENT RATIO

invitro RELEASE MEDIUM	TIME IN HOURS	BATCH CODE(SURFACTANT:CHOLESTEROL)			
		F5(3:1)	F6(4:1)	F7(5:1)	F8(6:1)
		MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD
PBS pH 7.4	0.25	3.0±0.65	1.9±0.44	1.1±0.16	1.2±0.21
	0.50	5.5±0.43	3.2±0.20	3.1±0.20	2.4±0.24
	0.75	7.8±0.37	4.3±0.41	4.5±0.24	3.4±0.16
	1	9.7±0.33	6.4±0.41	5.8±0.29	5.1±0.24
	1.5	11.9±0.66	8.5±0.21	8.1±0.20	6.4±0.24
	2	14.4±0.50	11.0±0.12	10.4±0.37	8.1±0.46
	2.5	16.7±0.54	13.5±0.40	12.0±0.20	10.2±0.67
	3	18.8±0.55	16.2±0.16	15.1±1.13	12.4±0.38
	3.5	21.6±0.08	19.0±0.24	18.1±0.24	15.0±0.35
	4	24.2±0.43	22.0±0.04	21.1±0.25	17.0±0.36
	4.5	27.1±0.35	24.6±0.32	24.2±0.33	19.7±0.37
	5	30.5±0.62	28.3±0.55	27.9±1.32	22.4±0.40
	5.5	34.5±0.26	30.6±0.33	29.9±0.33	25.5±0.57
	6	39.0±0.72	34.1±0.32	32.2±0.33	28.0±0.16
	6.5	42.3±0.73	37.7±0.50	35.5±0.32	31.6±0.16
	7	45.6±0.67	41.3±0.62	38.4±0.44	35.6±0.16
	7.5	48.7±0.69	45.0±0.45	41.4±0.32	38.1±0.53
	8	51.9±0.44	48.7±0.14	44.4±0.26	41.2±0.28
	8.5	55.3±0.23	52.9±0.42	47.3±0.49	44.7±0.57
	9	58.6±0.16	56.4±0.37	51.3±0.36	47.8±0.57
	9.5	62.4±0.33	60.4±0.32	55.2±0.49	51.8±0.49
	10	67.4±0.47	64.5±0.20	58.9±0.28	55.5±0.42
	10.5	71.5±0.68	68.2±0.86	62.6±1.66	59.5±0.40
	11	76.3±0.37	73.4±0.71	67.4±0.95	64.2±0.28
	11.5	81.7±0.36	76.4±0.71	71.9±0.68	68.8±0.59
	12	85.3±0.46	83.6±0.82	77.6±0.82	74.2±0.75

n=3*

TABLE VIIc *invitro* CUMULATIVE % DRUG RELEASE PROFILE OF NIOSOME CONTAINING SPAN-60 IN DIFFERENT RATIO

***invitro* RELEASE MEDIUM**	**TIME IN HOURS**	**BATCH CODE(SURFACTANT:CHOLESTEROL)**			
		F9(3:1)	**F10(4:1)**	**F11(5:1)**	**F12(6:1)**
		MEAN±SD	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
PBS pH 7.4	0.25	2.6±0.20	1.7±0.20	1.1±0.21	0.76±0.12
	0.50	4.5±0.16	3.4±0.16	2.1±0.16	1.6±0.24
	0.75	6.7±0.24	5.5±0.29	3.0±0.09	2.4±0.20
	1	9.0±0.36	7.7±0.45	4.7±0.16	3.3±0.24
	1.5	11.4±0.20	9.5±0.14	6.6±0.12	4.6±0.24
	2	13.9±0.20	11.4±0.21	8.4±0.28	6.4±0.16
	2.5	16.5±0.32	13.6±0.21	10.1±0.44	7.7±0.28
	3	19.3±0.37	16.4±0.24	12.1±0.37	9.7±0.28
	3.5	22.8±0.26	18.3±0.12	14.3±0.16	11.6±0.32
	4	26.1±0.26	20.4±0.36	16.7±0.18	13.6±0.35
	4.5	29.5±0.20	23.3±0.28	18.9±0.26	15.8±0.49
	5	32.7±0.24	26.0±0.26	21.3±0.21	18.1±0.40
	5.5	35.5±0.12	28.9±0.33	24.0±0.16	20.5±0.21
	6	39.2±0.63	31.7±0.20	26.3±0.20	22.7±0.32
	6.5	41.6±0.44	34.5±0.21	28.8±0.32	25.0±0.24
	7	44.3±0.41	37.3±0.33	31.5±0.26	27.2±0.24
	7.5	47.6±0.14	40.2±0.46	33.9±0.98	29.9±0.63
	8	51.1±0.24	43.1±0.41	36.4±0.33	32.5±0.54
	8.5	54.9±0.44	45.8±0.24	39.1±0.26	34.8±0.62
	9	58.2±0.64	49.0±0.16	41.6±0.26	37.6±0.49
	9.5	61.7±0.70	52.3±0.28	44.6±0.36	41.0±0.30
	10	66.0±0.94	55.3±0.20	47.2±0.16	43.7±0.45
	10.5	69.5±1.06	58.9±0.20	50.7±0.20	46.2±0.44
	11	72.9±1.20	62.8±0.31	54.5±0.12	49.5±0.45
	11.5	75.9±1.26	66.2±0.42	57.4±0.97	53.0±0.32
	12	78.5±1.16	69.8±0.21	62.2±0.41	55.3±1.02

n=3*

TABLE VIId *invitro* CUMULATIVE % DRUG RELEASE PROFILE OF NIOSOME CONTAINING SPAN-80 IN DIFFERENT RATO

***invitro* RELEASE MEDIUM**	**TIME IN HOURS**	**BATCH CODE(SURFACTANT:CHOLESTEROL)**			
		F13(3:1)	**F14(4:1)**	**F15(5:1)**	**F16(6:1)**
		MEAN±SD	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
PBS pH 7.4	0.25	6.0±0.33	4.8±0.26	4.1±0.24	2.3±0.75
	0.50	9.3±0.32	6.8±0.30	5.7±0.32	4.6±0.20
	0.75	12.1±0.14	9.2±0.21	8.0±0.08	7.8±0.35
	1	15.2±0.35	11.1±0.20	9.9±0.36	9.3±0.16
	1.5	18.1±0.16	13.7±0.24	13.0±0.09	12.0±0.28
	2	21.0±0.32	13.7±0.24	15.5±0.21	14.7±0.20
	2.5	23.9±0.37	16.2±0.16	17.0±0.35	16.7±0.63
	3	27.1±0.28	17.7±0.43	20.1±0.28	19.1±0.58
	3.5	29.9±0.32	21.2±0.21	23.0±0.36	22.0±0.38
	4	32.8±0.36	23.8±0.12	25.3±0.37	24.0±0.23
	4.5	35.8±0.12	26.3±0.16	28.0±0.28	27.0±0.44
	5	39.0±0.16	28.9±0.12	30.8±0.18	29.4±0.57
	5.5	42.2±0.50	33.9±0.29	33.4±0.09	32.5±0.69
	6	46.2±0.37	34.7±0.83	35.8±0.21	35.3±0.37
	6.5	50.1±0.26	36.6±0.29	38.9±0.20	37.5±0.28
	7	54.3±0.26	40.3±0.24	42.1±0.85	40.3±0.17
	7.5	57.9±0.16	43.9±0.20	46.0±0.87	43.6±0.23
	8	64.4±0.28	47.1±0.35	50.5±1.40	48.5±0.19
	8.5	68.1±0.36	51.0±0.14	55.0±1.06	52.4±0.16
	9	71.9±0.16	55.4±0.16	60.2±0.69	57.1±0.41
	9.5	76.0±0.21	59.9±0.46	64.0±0.92	61.0±0.47
	10	80.4±0.16	64.5±0.24	68.2±0.67	66.2±0.48
	10.5	84.6±0.18	69.7±0.33	73.8±1.24	70.9±0.69
	11	89.0±0.04	74.1±0.12	78.2±1.20	75.7±0.36
	11.5	92.2±0.24	83.8±0.38	83.0±0.61	80.4±0.20
	12	96.1±0.35	89.9±0.26	87.3±0.94	86.0±0.37

n=3*

TABLE VIIe *invitro* CUMULATIVE % DRUG RELEASE PROFILE OF NIOSOME CONTAINING TWEEN-60 IN DIFFERENT RATIO

***invitro* RELEASE MEDIUM**	**TIME IN HOURS**	**BATCH CODE(SURFACTANT:CHOLESTEROL)**			
		F17(3:1)	**F18(4:1)**	**F19(5:1)**	**F20(6:1)**
		MEAN±SD	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
PBS pH 7.4	0.25	6.1±0.28	5.2±0.24	5.0±0.12	3.9±0.16
	0.50	8.3±0.21	6.0±0.16	6.1±0.12	5.1±0.16
	0.75	10.2±0.12	8.4±0.24	7.8±0.24	6.8±0.73
	1	12.4±0.08	10.4±0.16	9.8±0.08	8.7±0.12
	1.5	15.0±0.16	11.4±0.69	11.3±0.01	10.3±0.12
	2	17.5±0.16	14.3±0.16	13.9±.16	12.1±0.12
	2.5	20.0±0.28	16.4±0.16	15.8±0.08	14.1±0.20
	3	23.2±0.28	18.7±0.26	17.9±0.29	16.5±0.24
	3.5	27.0±0.16	21.3±0.17	20.4±0.16	18.3±0.16
	4	29.9±0.32	23.9±0.21	22.5±0.12	20.5±0.16
	4.5	33.7±0.28	26.6±0.94	24.7±0.12	22.8±0.20
	5	36.1±0.24	29.1±0.09	26.8±0.21	25.0±0.16
	5.5	39.8±0.29	32.4±0.16	29.4±0.16	27.2±0.16
	6	42.0±0.41	35.6±0.18	32.7±0.18	29.7±0.12
	6.5	46.0±0.32	38.8±0.29	36.1±0.21	32.8±0.41
	7	49.6±0.44	42.2±0.36	38.2±0.16	35.5±0.26
	7.5	53.6±0.41	46.8±0.16	41.5±0.08	38.6±0.29
	8	57.4±0.15	51.5±0.08	44.5±0.24	41.4±0.169
	8.5	61.4±0.24	56.8±0.20	48.3±0.24	44.8±0.12
	9	65.9±0.38	61.1±0.23	52.5±0.14	48.4±0.28
	9.5	70.4±0.16	65.3±0.30	56.5±0.04	52.0±0.08
	10	75.5±0.67	70.5±0.14	61.5±0.28	55.9±0.16
	10.5	80.3±0.47	75.6±0.09	66.5±0.28	60.9±0.16
	11	85.03±0.47	78.7±.30	71.6±0.29	65.3±0.29
	11.5	87.1±0.74	83.2±0.16	76.5±0.53	70.2±0.70
	12	94.0±0.41	87.5±0.04	82.7±1.11	76.4±0.16

n=3*

TABLE VIIf ***invitro*** **CUMULATIVE % DRUG RELEASE PROFILE OF NIOSOME CONTAINING TWEEN-80 IN DIFFERENT RATIO**

invitro **RELEASE MEDIUM**	**TIME IN HOURS**	**BATCH CODE(SURFACTANT:CHOLESTEROL)**			
		F21(3:1)	**F22(4:1)**	**F23(5:1)**	**F24(6:1)**
		MEAN±SD	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
PBS pH 7.4	0.25	6.8±0.24	5.9±0.32	4.5±0.24	3.8±0.16
	0.50	8.9±0.24	8.2±0.20	6.3±0.20	5.4±0.12
	0.75	12.5±0.47	10.2±0.29	8.0±0.12	7.0±0.32
	1	14.8±0.61	11.4±0.24	10.0±0.16	8.7±0.24
	1.5	17.8±0.54	14.1±0.28	12.2±0.20	10.4±0.32
	2	21.0v0.83	15.8±0.18	14.1±0.20	12.4±0.16
	2.5	24.1±0.73	17.9±0.30	16.3±0.14	14.2±0.16
	3	27.3±0.53	20.9±0.86	18.5±0.14	16.0±0.12
	3.5	29.9±0.49	23.9±0.53	21.0±0.12	18.6±0.21
	4	32.2±1.02	26.7±0.46	23.4±0.21	21.1±0.23
	4.5	35.1±0.52	29.6±0.49	25.8±0.16	23.3±0.21
	5	38.7±0.57	32.5±0.91	29.0±0.20	25.9±0.16
	5.5	41.6±0.54	35.4±0.30	31.4±0.11	28.5±0.30
	6	44.9±0.49	39.8±0.73	34.1±0.12	31.2±0.16
	6.5	48.1±0.50	43.2±0.89	38.0±0.20	33.5±0.12
	7	51.2±0.63	46.4±0.74	41.5±0.16	36.4±0.12
	7.5	54.7±0.57	50.2±1.06	46.2±0.16	39.3±0.32
	8	58.3±0.89	54.8±1.06	50.4±0.11	42.5±0.29
	8.5	62.5±0.97	57.8±0.61	54.8±0.26	45.8±0.21
	9	66.8±1.11	61.7±0.83	59.2±0.28	48.7±0.32
	9.5	72.0±0.61	66.7±0.63	64.1±0.32	52.7±0.98
	10	77.6±0.81	70.80.55	68.3±0.32	58.6±0.18
	10.5	82.2±0.50	76.0±0.92	73.1±0.08	62.7±0.30
	11	88.3±0.50	79.5±0.50	76.5±0.16	66.9±0.36
	11.5	92.4±0.36	85.2±0.95	80.2±0.20	71.9±0.36
	12	96.0±0.42	90.4±1.09	83.6±0.57	76.1±0.23

n=3*

TABLE VIIg *invitro* CUMULATIVE% DRUG RELEASE PROFILE OF NIOSOME CONTAINING BRIJ-52 IN DIFFERENT RATIO

***invitro* RELEASE MEDIUM**	**TIME IN HOURS**	**BATCH CODE(SURFACTANT:CHOLESTEROL)**			
		F25(3:1)	**F26(4:1)**	**F27(5:1)**	**F28(6:1)**
		MEAN±SD	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
PBS PH 7.4	0.25	5.1±0.04	4.1±0.24	3.4±0.46	2.9±0.17
	0.50	7.0±**0.16**	6.0±0.16	4.0±0.24	4.2±0.28
	0.75	8.7±0.24	7.6±0.16	6.3±0.31	5.8±0.37
	1	10.9±0.12	9.6±0.24	7.9±0.46	7.5±0.36
	1.5	13.4±0.16	11.3±0.62	9.9±0.52	8.9±0.66
	2	15.5±0.08	13.6±0.32	11.9±0.60	10.8±0.33
	2.5	17.5±0.21	16.0±0.36	13.9±0.46	12.2±0.06
	3	20.8±0.20	17.7±0.44	15.8±0.36	14.5±0.07
	3.5	23.3±0.16	19.4±0.33	17.8±0.42	16.4±0.15
	4	26.1±0.23	22.8±0.20	19.8±0.56	18.4±0.46
	4.5	29.0±0.16	25.8±0.20	22.1±0.71	20.0±0.39
	5	32.3±0.18	27.0±0.16	24.3±0.33	22.4±0.99
	5.5	34.6±0.29	30.2±0.14	27.6±0.48	24.7±0.16
	6	37.4±0.17	32.4±0.14	31.06±0.42	27.7±0.16
	6.5	40.2±0.36	35.8±0.17	35.3±0.51	29.2±0.12
	7	43.3±0.272	38.7±0.46	37.7±0.66	31.2±0.18
	7.5	47.4±0.96	41.4±0.17	40.0±0.43	34.0±0.19
	8	50.4±0.26	44.7±0.26	43.3±0.51	36.0±0.09
	8.5	53.9±0.47	48.9±0.21	46.6±0.28	39.6±0.10
	9	56.2±0.16	51.5±0.12	49.8±0.33	42.0±0.10
	9.5	61.0±0.65	55.2±0.02	53.4±0.16	44.0±0.24
	10	65.7±0.72	59.2±0.12	57.4±0.06	49.3±0.51
	10.5	68.3±0.16	62.0±0.17	61.3±0.06	53.4±0.21
	11	72.3±0.12	66.9±0.27	64.5±0.16	57.9±0.36
	11.5	76.4±0.17	71.2±0.16	68.8±0.19	62.3±0.52
	12	81.3±0.16	75.3±0.14	74.1±0.86	66.7±0.12

n=3*

TABLE V111a ***invitro*** **RELEASE KINETICS OF ETORICOXIB NIOSOMES CONTAINING SPAN-20 IN DIFFERENT RATIO**

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
SPAN-20	R^2	$KO^{(h-1)}$	R^2	$K1^{(h-1)}$	R^2	$KH^{h(-1/2)}$	R^2	n value	R^2	$KHC^{(h-1/3)}$
F1 (3:1)	0.991	6.741	0.896	-0.058	0.928	27.29	0.980	0.929	0.921	-0.161
F2 (4:1)	0.994	6.965	0.880	-0.064	0.942	27.39	0.978	0.860	0.939	-0.174
F3 (5:1)	0.993	6.749	0.891	-0.057	0.947	27.41	0.986	0.949	0.942	-0.160
F4 (6:1)	0.983	6.612	0.885	-0.053	0.911	26.59	0.973	0.990	0.929	-0.152

TABLE VIIIb *invitro* RELEASE KINETICS OF ETORICOXIB NIOSOMES CONTAINING SPAN-40 IN DIFFERENT RATIO

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
SPAN-40	**R^2**	**$KO^{(h-1)}$**	**R^2**	**$K1^{(h-1)}$**	**R^2**	**$KH^{h(-1/2)}$**	**R^2**	**n value**	**R^2**	**$KHC^{(h-1/3)}$**
F5 (3:1)	0.991	6.741	0.896	-0.058	0.949	27.17	0.985	0.849	0.941	-0.161
F6 (4:1)	0.989	6.695	0.899	-0.053	0.921	27.06	0.991	1.068	0.941	-0.153
F7 (5:1)	0.991	6.156	0.917	-0.045	0.933	24.92	0.994	1.061	0.951	-0.134
F8 (6:1)	0.983	5.970	0.913	-0.042	0.905	23.98	0.987	1.140	0.943	-0.126

Table VIII c ***invitro*** **RELEASE KINETICS OF ETORICOXIB NIOSOMES CONTAINING SPAN-60 AT DIFFERENT RATIO**

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
SPAN-60	R^2	$KO^{(h-1)}$	R^2	$K1^{(h-1)}$	R^2	$KH^{h(-1/2)}$	R^2	n value	R^2	$KHC^{(h-1/3)}$
F9 (3:1)	0.998	6.426	0.949	-0.051	0.953	26.27	0.992	0.922	0.974	-0.147
F10 (4:1)	0.995	5.581	0.951	-0.039	0.938	22.69	0.986	0.938	0.971	-0.117
F11 (5:1)	0.992	4.984	0.955	-0.031	0.929	20.19	0.994	0.961	0.971	-0.099
F12 (6:1)	0.989	4.622	0.958	-0.027	0.998	26.23	0.996	0.878	0.971	-0.088

TABLE VIII d *invitro* RELEASE KINETICS OF ETORICOXIB NIOSOMES CONTAINING SPAN-80 AT DIFFERENT RATIO

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
SPAN-80	R^2	$KO^{(h-1)}$	R^2	$K1^{(h-1)}$	R^2	$KH^{h(-1/2)}$	R^2	n value	R^2	$KHC^{(h-1/3)}$
F13 (3:1)	0.994	7.476	0.834	-0.088	0.941	30.43	0.974	0.795	0.922	-0.214
F14 (4:1)	0.982	6.781	0.843	-0.062	0.909	27.31	0.970	0.872	0.908	-0.169
F15 (5:1)	0.982	6.771	0.865	-0.060	0.910	27.27	0.973	0.902	0.919	-0.165
F16 (6:1)	0.981	6.602	0.865	-0.056	0.910	27.12	0.975	0.913	0.918	-0.157

TABLE VIII e *invitro* RELEASE KINETICS OF ETORICOXIB NIOSOMES CONTAINING TWEEN-60 AT DIFFERENT RATIO

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
TWEEN-60	R^2	$KO^{(h-1)}$	R^2	$K1^{(h-1)}$	R^2	$KH^{h(-1/2)}$	R^2	n value	R^2	$KHC^{(h-1/3)}$
F17 (3:1)	0.992	7.200	0.837	-0.074	0.929	27.31	0.979	0.859	0.917	-0.192
F18 (4:1)	0.980	6.920	0.870	-0.062	0.900	27.75	0.954	0.783	0.920	-0.169
F19 (5:1)	0.973	6.128	0.862	-0.049	0.899	23.34	0.956	0.752	0.910	-0.141
F20 (6:1)	0.975	5.678	0.889	-0.041	0.896	22.77	0.966	0.784	0.926	-0.123

TABLE VIII f ***invitro*** **RELEASE KINETICS OF ETORICOXIB NIOSOMES CONTAINING TWEEN-80 AT DIFFERENT RATIO**

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
SPAN-80	R^2	$KO^{(h-1)}$	R^2	$K1^{(h-1)}$	R^2	$KH^{h(-1/2)}$	R^2	n value	R^2	$KHC^{(h-1/3)}$
F21 (3:1)	0.988	7.201	0.791	-0.088	0.929	29.21	0.976	0.693	0.892	-0.204
F22 (4:1)	0.982	6.677	0.893	-0.056	0.934	26.82	0.963	0.786	0.932	-0.159
F23 (5:1)	0.982	6.677	0.893	-0.054	0.904	26.89	0.959	0.792	0.941	-0.147
F24 (6:1)	0.978	5.797	0.896	-0.043	0.900	23.27	0.967	0.788	0.931	-0.127

TABLE VIIIg ***invitro*** **RELEASE KINETICS OF ETORICOXIB NIOSOMES CONTAINING BRIJ-52 IN DIFFERENT RATIO**

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
BRIJ-52	**R^2**	**$KO^{(h-1)}$**	**R^2**	**$K1^{(h-1)}$**	**R^2**	**$KH^{h(-1/2)}$**	**R^2**	**n value**	**R^2**	**$KHC^{(h-1/3)}$**
F25 (3:1)	0.993	6.212	0.935	-0.045	0.935	25.22	0.976	0.740	0.955	-0.144
F26 (4:1)	0.988	5.763	0.923	-0.043	0.904	26.82	0.963	0.786	0.932	-0.159
F27 (5:1)	0.986	5.761	0.924	-0.041	0.914	23.21	0.973	0.825	0.951	-0.124
F28 (6:1)	0.977	5.000	0.915	-0.033	0.901	20.10	0.975	0.810	0.941	-0.102

TABLE IX a COMPOSITION OF ETORICOXIB PLAIN GEL

SL. NO	INGREDIENTS	FOR 50 G
1.	Carbopol 934	0.9g
2.	Triethanolamine	0.5 ml
3.	Water	50 ml
4.	Drug (ETORICOXIB)	500 mg

TABLE IX b COMPOSITION OF ETORICOXIB NIOSOMAL GEL

SL. NO	INGREDIENTS	FOR 50 G
1.	Carbopol 934	0.9g
2.	Triethanolamine	0.5 ml
3.	Water	50 ml
4.	Etoricoxib Niosomes	25 ml

TABLE X DRUG CONTENT OF ETORICOXIB NIOSOMAL GEL

S.NO	FORMULATION	% DRUG CONTENT
1	FG12(SPAN 60) 6:1	98.70
2	FG21(TWEEN80) 3:1	99.02
3	PLAIN ETORICOXIB GEL	98.88

TABLE XI APPEARANCE, CLARITY AND pH OF FORMULATIONS

FORMULATIONS	APPEARANCE	CLARITY	pH at 0 day	pH at 15 th day	pH at 30 th day
Plain gel	Colourless	Clear	7.0	6.9	6.9
FG12 SPAN60 (6:1)	Colourless	Clear	6.9	6.9	6.9
FG21 TWEEN 80 (3:1)	Colourless	Clear	7.0	7.0	7.0

TABLE XII RHEOLOGICAL EVALUATION OF NIOSOMAL GEL FORMULATIONS

S.NO	RPM	VISCOSITY IN CPS	
		FG12	FG21
1	0.1	54888	44091
2	0.5	11937	9178
3	1.0	5699	4499
4	5.0	1188	935
5	10.0	666	542
6	20.0	415	325
7	50.0	202	172
8	100	132	114

n=3*

TABLE XIII COMPARISON OF *invitro* CUMULATIVE % DRUG RELEASE PROFILE OF PLAIN GEL AND NIOSOMEL GEL CONTAINING HIGH ENTRAPMENT AND LOW ENTRAPMENT

***invitro* RELEASE MEDIUM**	**TIME IN HOURS**	**BATCH CODE(SURFACTANT:CHOLESTEROL)**		
		FG12 (6:1):(H.E)	**FG21 (3:1):(L.E)**	**PLAIN GE;L**
		MEAN±SD	**MEAN±SD**	**MEAN±SD**
PBS pH 7.4	0.25	0.89±0.36	7.0±0.41	9.2±0.16
	0.50	1.58±0.42	8.8±0.28	17.6±0.16
	0.75	2.7±0.51	11.9±0.28	22.9±0.46
	1	3.5±0.65	15.6±0.36	30.4±0.32
	1.5	4.4±0.73	19.5±0.41	36.1±0.28
	2	5.8±0.21	23.4±0.57	41.2±0.73
	2.5	7.1±0.33	26.1±0.72	45.0±0.87
	3	8.9±0.49	29.4±0.86	51.9±0.96
	3.5	12.7±0.51	31.5±0.73	57.1±0.21
	4	14.5±0.21	34.8±0.16	62.5±0.16
	4.5	16.9±0.38	39.4±0.28	69.1±0.71
	5	17.8±0.16	42.6±0.63	76.7±0.71
	5.5	23.7±0.29	45.4±0.45	80.5±0.86
	6	28.5±0.38	47.9±0.41	90.9±0.93
	6.5	30.9±0.45	49.2±0.67	95.1±0.16
	7	32.2±0.21	52.4±0.17	98.8±0.58
	7.5	35.5±0.16	55.8±0.46	
	8	36.7±0.43	59.3±0.71	
	8.5	39.3±0.26	62.8±0.21	
	9	42.2±0.21	66.5±0.48	
	9.5	45.1±0.86	71.2±0.51	
	10	48.4±0.21	76.1±0.49	
	10.5	49.7±0.86	83.1±0.53	
	11	53.1±0.16	88.9±0.51	
	11.5	55.4±0.93	92.1±0.48	
	12	58.8±0.86	95.1±0.37	

n=3*

TABLE XIV *invitro* RELEASE KINETICS OF ETORICOXIB NIOSOMAL GEL CONTAININ FG12, FG21 AND PURE DRUG AT DIFFERENT RATIO

FORMULATION CODE	ZERO ORDER		FIRST ORDER		HIGUCHI MODEL		KORSMEYER-PEPPAS		HIXSON-CROWELL	
BRIJ-52	R^2	$K_O^{(h-1)}$	R^2	$K_1^{(h-1)}$	R^2	KH $^{h(-1/2)}$	R^2	n value	R^2	$KHC^{(h-1/3)}$
FG12(6:1)	0.991	5.080	0.971	-0.031	0.979	20.59	0.992	1.309	0.971	-0.099
FG21(3;1)	0.990	7.035	0.813	-0.813	0.944	28.85	0.979	0.765	0.904	-0.200
PURE DRUG	0.990	12.53	0.794	-0.204	0.980	40.65	0.977	0.634	0.929	-0.428

TABLE XV COMPARISON OF % INHIBITION OF RAT PAW EDEMA USING PLAIN ETORICOXIB GEL, NIOSOMAL GEL CONTAINING FG12 AND FG21

S.NO	TIME IN HOURS	STANDARD MEAN±SD	TEST-1 HIGH ENTRAPMENT(FG12) MEAN±SD	TEST-2 LOW ENTRAPMENT(FG21) MEAN±SD
1	1	28.2±0.72	16.9±0.82	27.7±0.70
2	2	28.9±0.82	19.9±0.98	28.4±0.78
3	3	34.8±0.52	18.1±0.68	31.2±0.70
4	4	39.8±0.50	19.1±0.46	37.3±0.56
5	5	49.0±0.52	19.4±0.69	38.9±0.48
6	6	51.7±0.44	21.2±0.62	44.5±0.32
7	24	59.6±0.30	23.9±0.46	46.0±0.52

n=3*

ONE WAY ANALYSIS OF VARIANCE (ANOVA)

COMPARSION GROUP	TIME INTERVALS			
	1st HOUR	3thHOUR	6th HOUR	24thHOUR
STD vs FG12	*** P<0.001	*** P<0.001	*** P<0.001	* P<0.05
STD vs FG21	*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001
FG12 vs FG12	*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001

n=3*

TABLE XVI a STABILITY STUDY ON ETORICOXIB CONTENT IN NIOSOMAL FORMULATION (FG12) FOR 3 MONTHS OF STORAGE PERIOD

REFRIGERATOR TEMPERATURE	TIME OF STORAGE IN MONTHS	DRUG CONTENT*
4^0C	0	98.62±0.23
	1	98.57±0.66
	2	98.23±0.25
	3	98.08±0.22

n=3*

TABLE 16 b STABILITY STUDY ON ETORICOXIB CONTENT IN NIOSOMAL FORMULATION (FG12) FOR 3 MONTHS OF STORAGE PERIOD

REFRIGERATOR TEMPERATURE	TIME OF STORAGE IN MONTHS	DRUG CONTENT*
4^0C	0	98.62±0.23
	1	94.57±0.66
	2	89.23±0.25
	3	86.08±0.22

n=3*

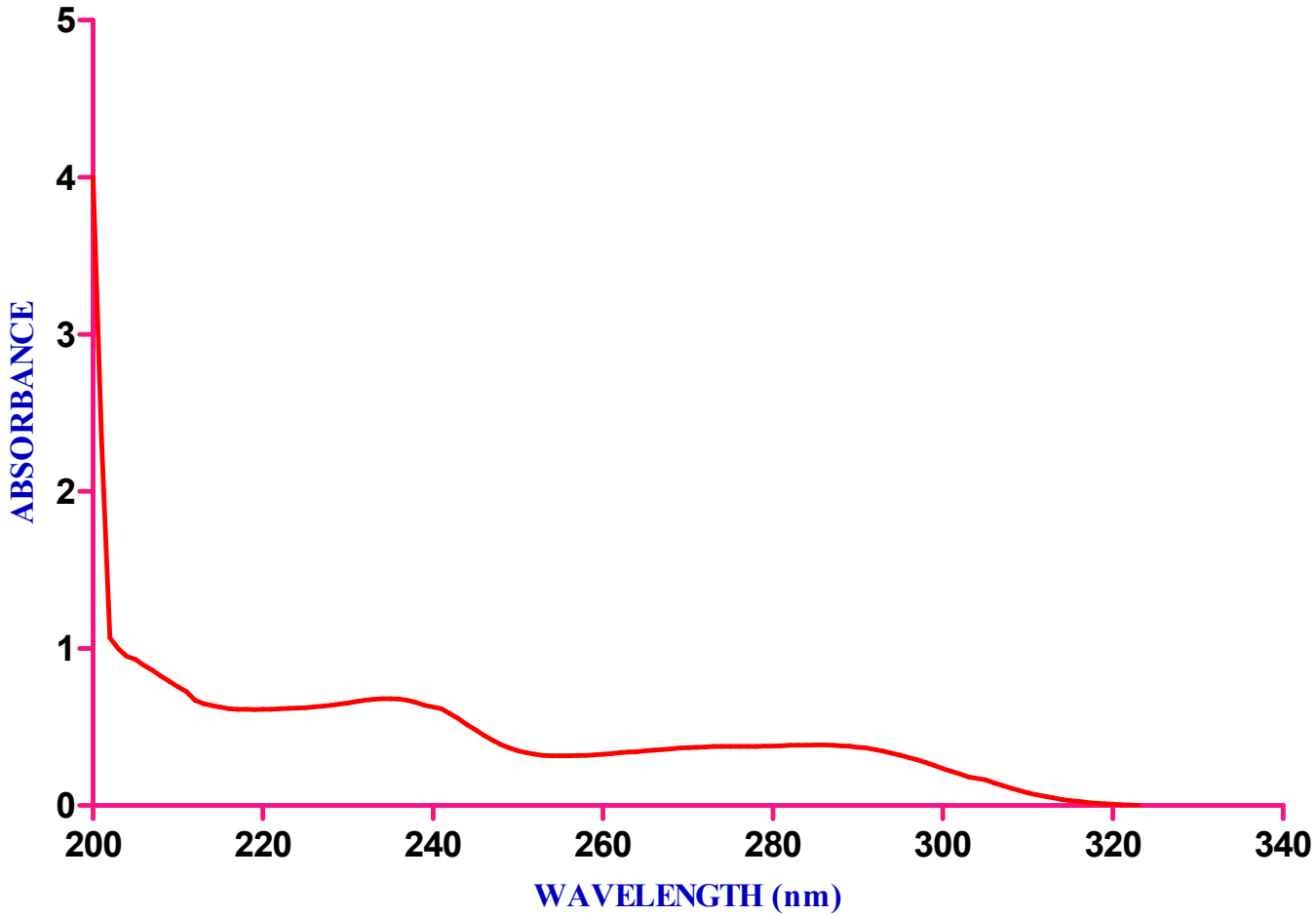


Figure 13 Determination of λmax of Etoricoxib

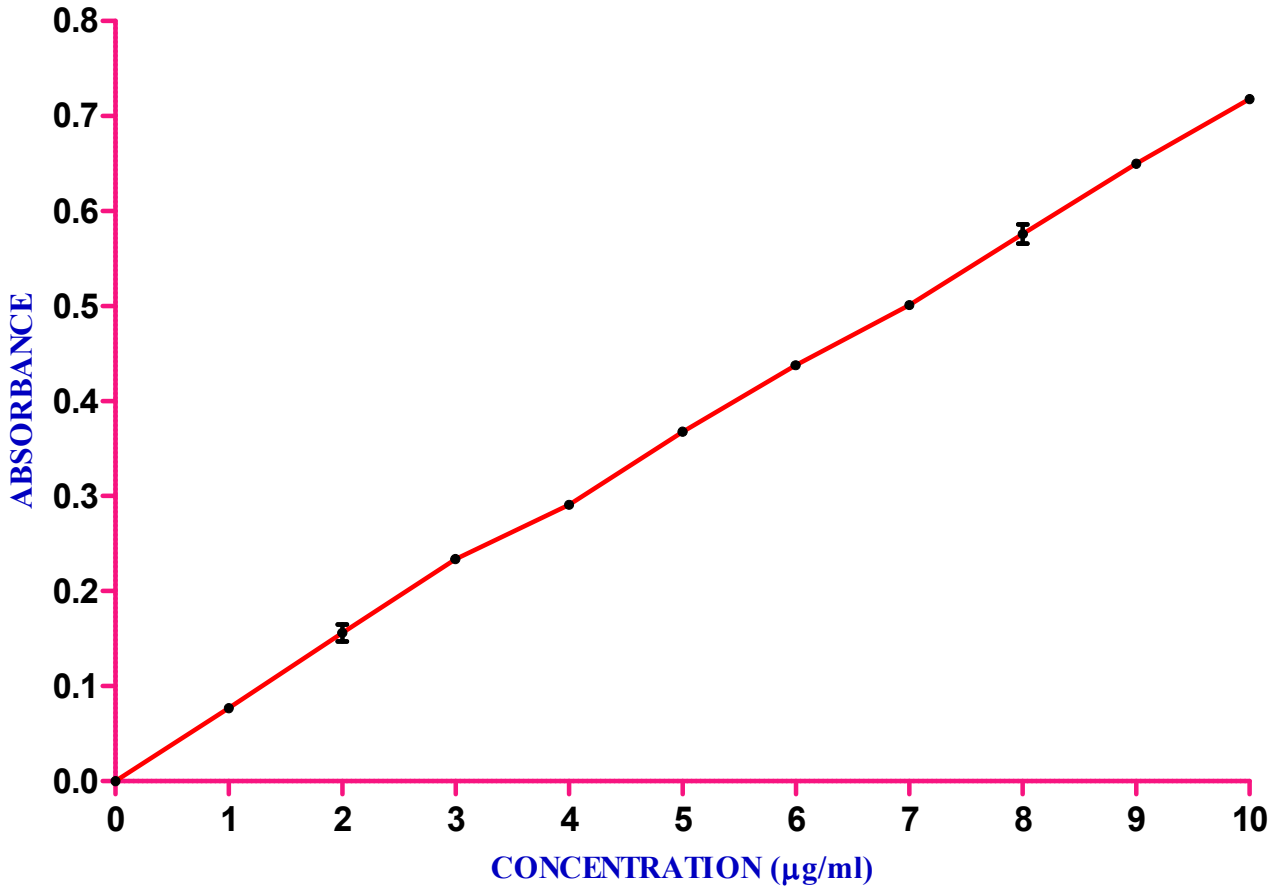
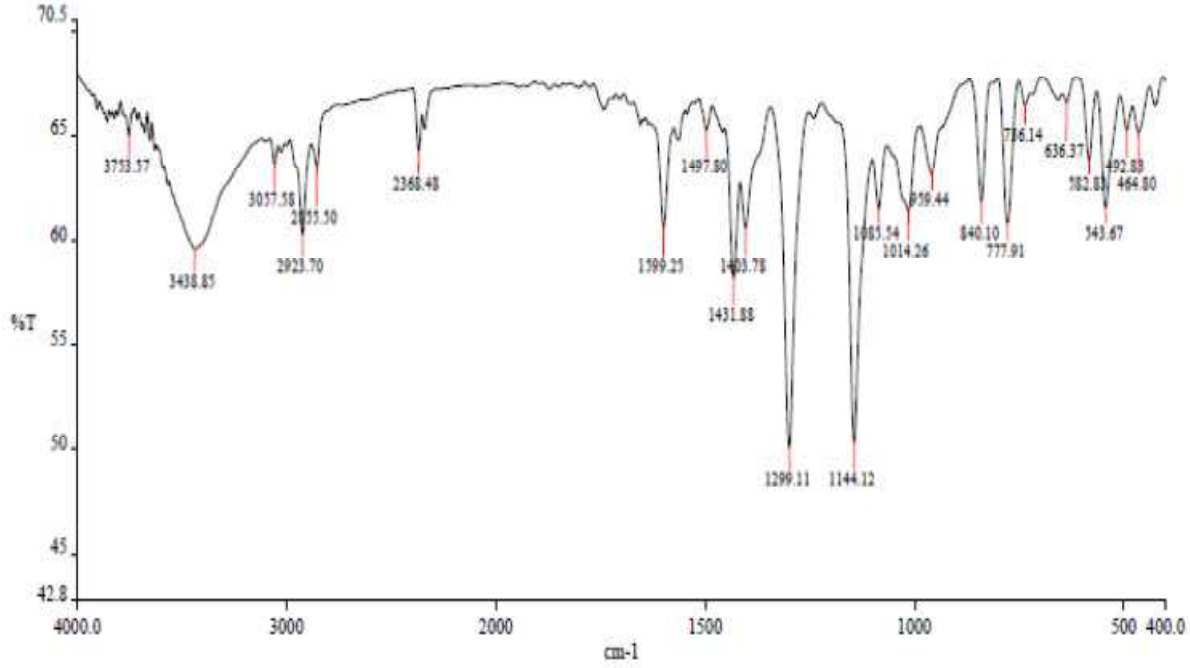


Figure 14 Calibration of Etoricoxib at Pbs of pH7.4

15(a) Etoricoxib



15(b) CHOLESTEROL

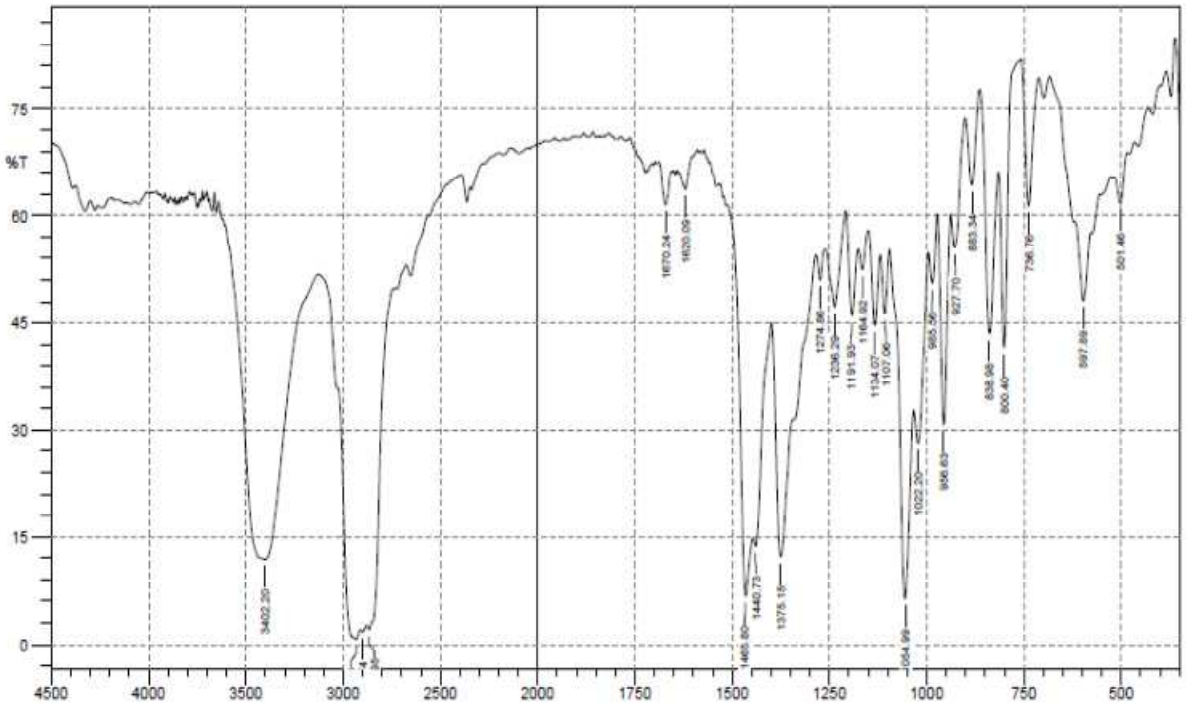
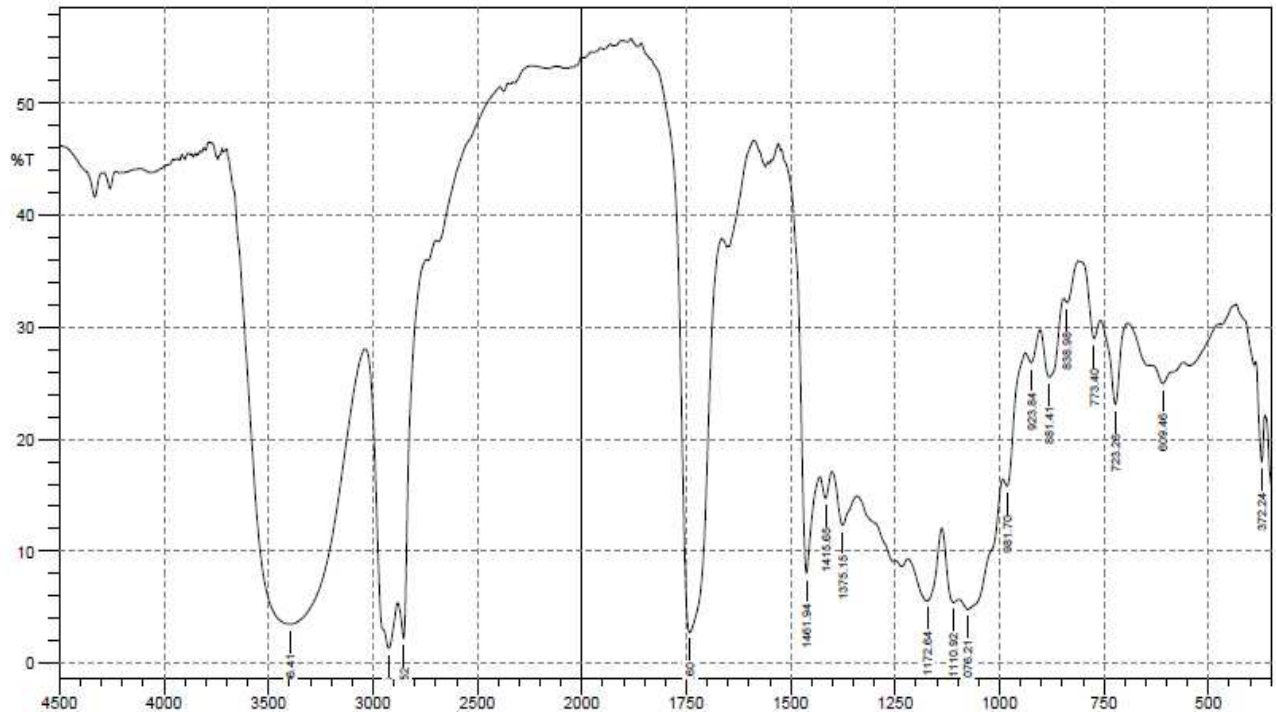


Figure 15a FT-IR spectra are as follows-(a) Etoricoxib (b) cholesterol

15(c) SPAN 20



15(d) SPAN 40

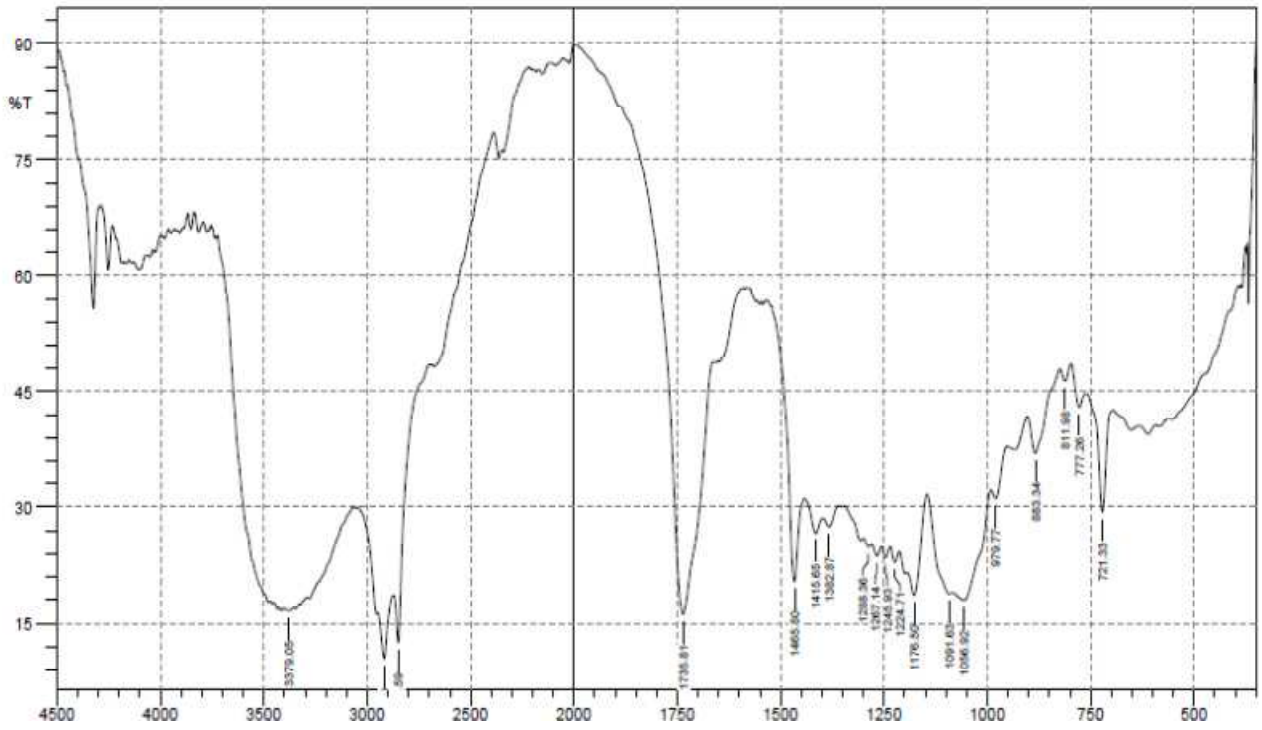
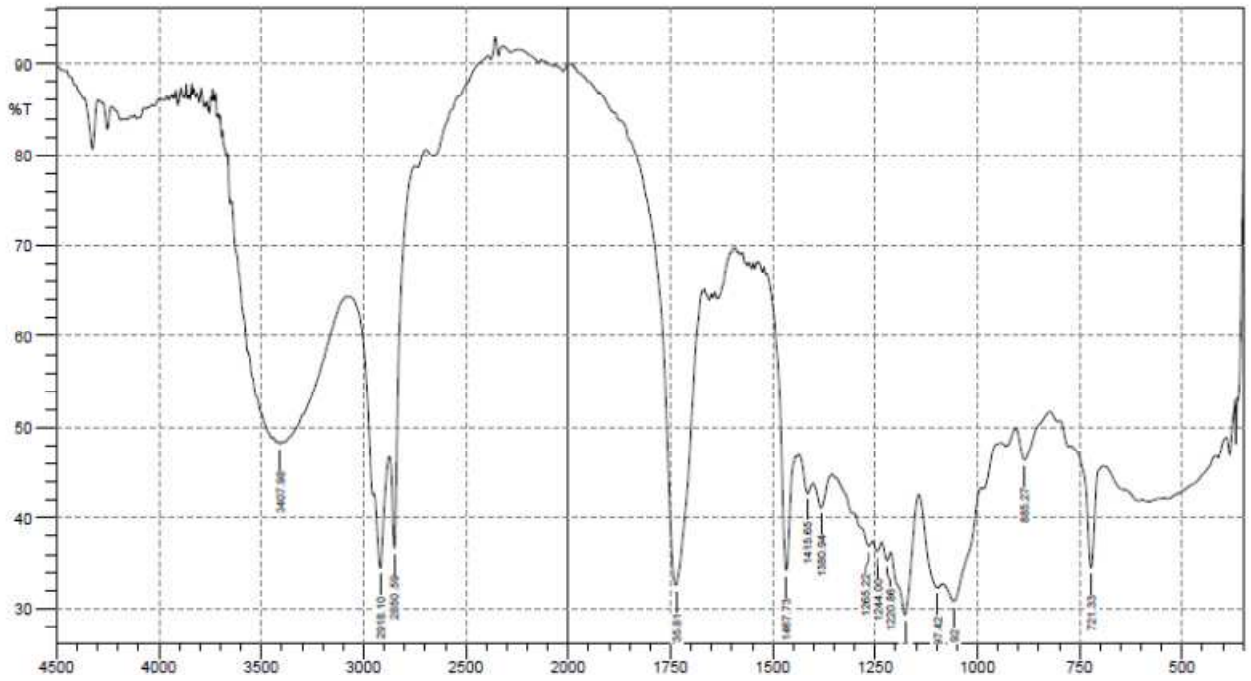


Figure 15b FT-IR spectra are as follows-(c) Span20, (d) Span40

15(e) SPAN 60



15(f) SPAN 80

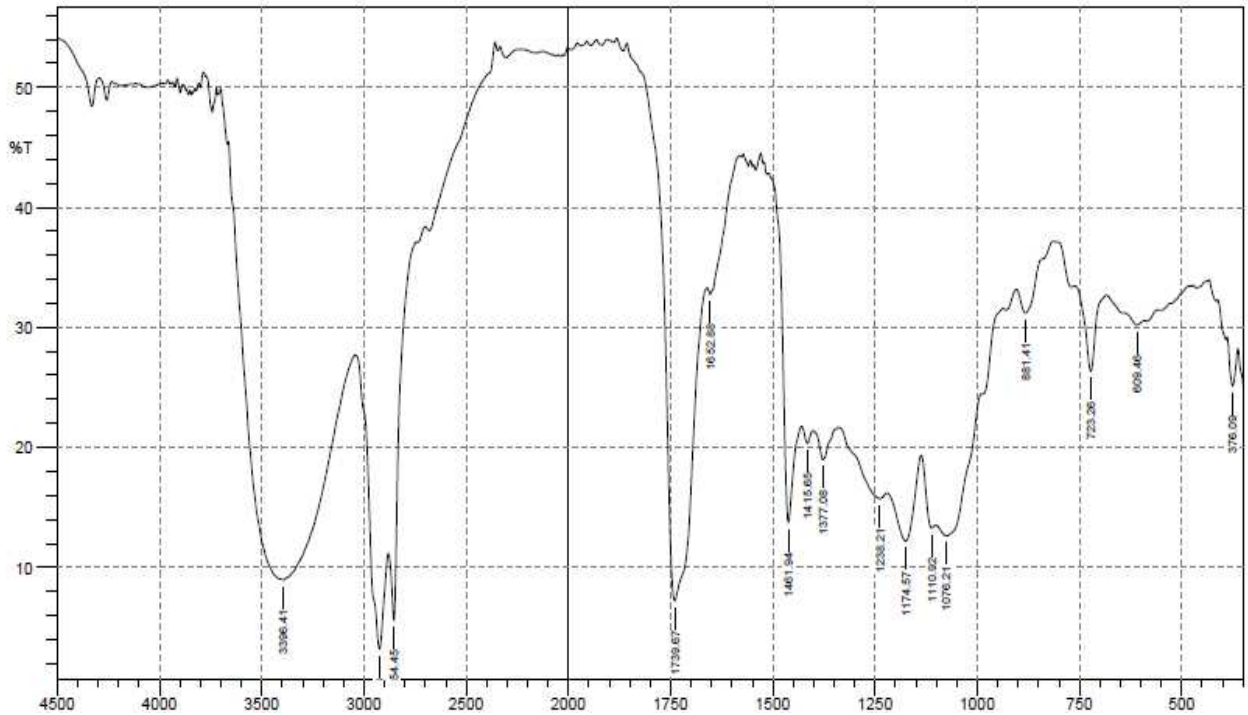
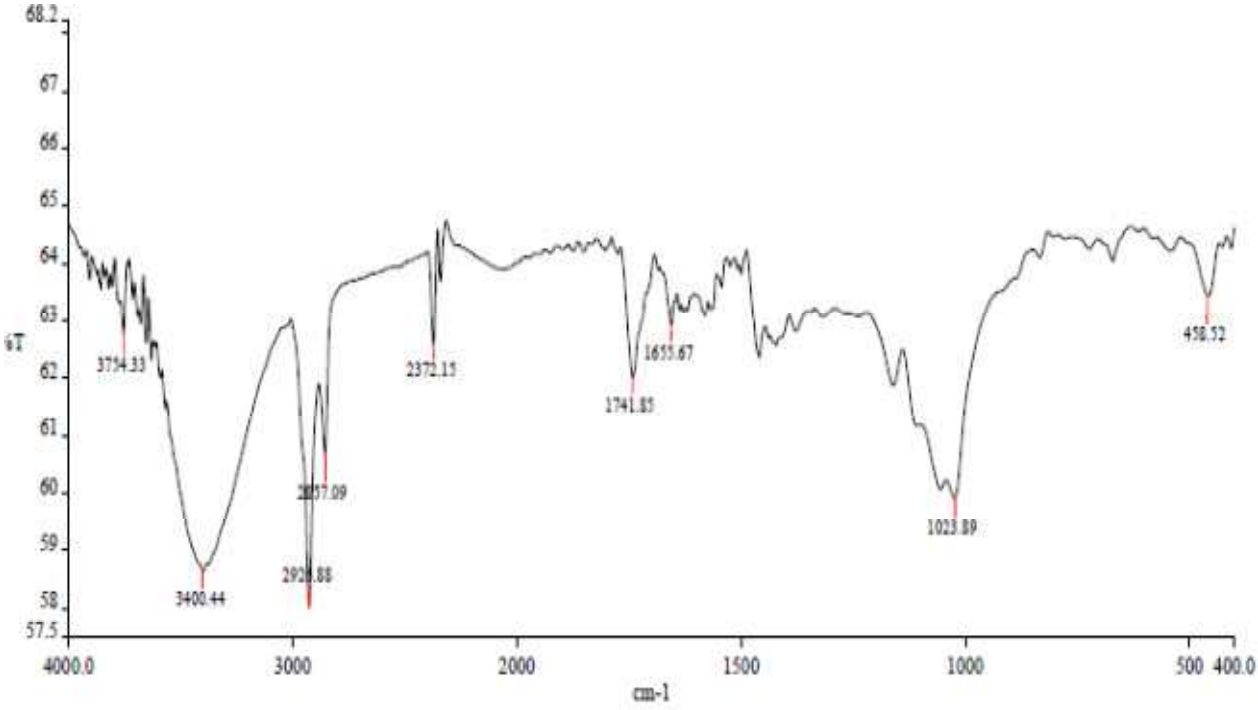


Figure 15c FT-IR spectra are as follows-(e) Span60, (f) Span80

15(g) SPAN 20 + DRUG + CHOLESTEROL



15(h) SPAN 40 + DRUG + CHOLESTEROL

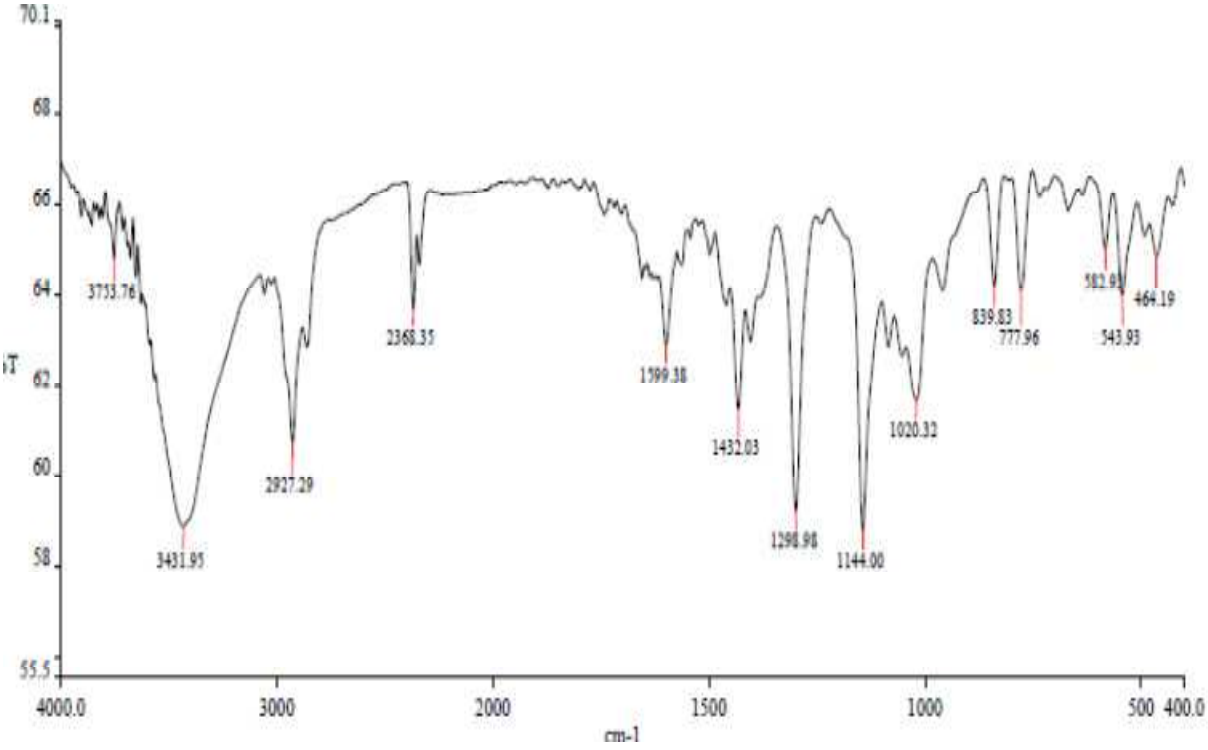
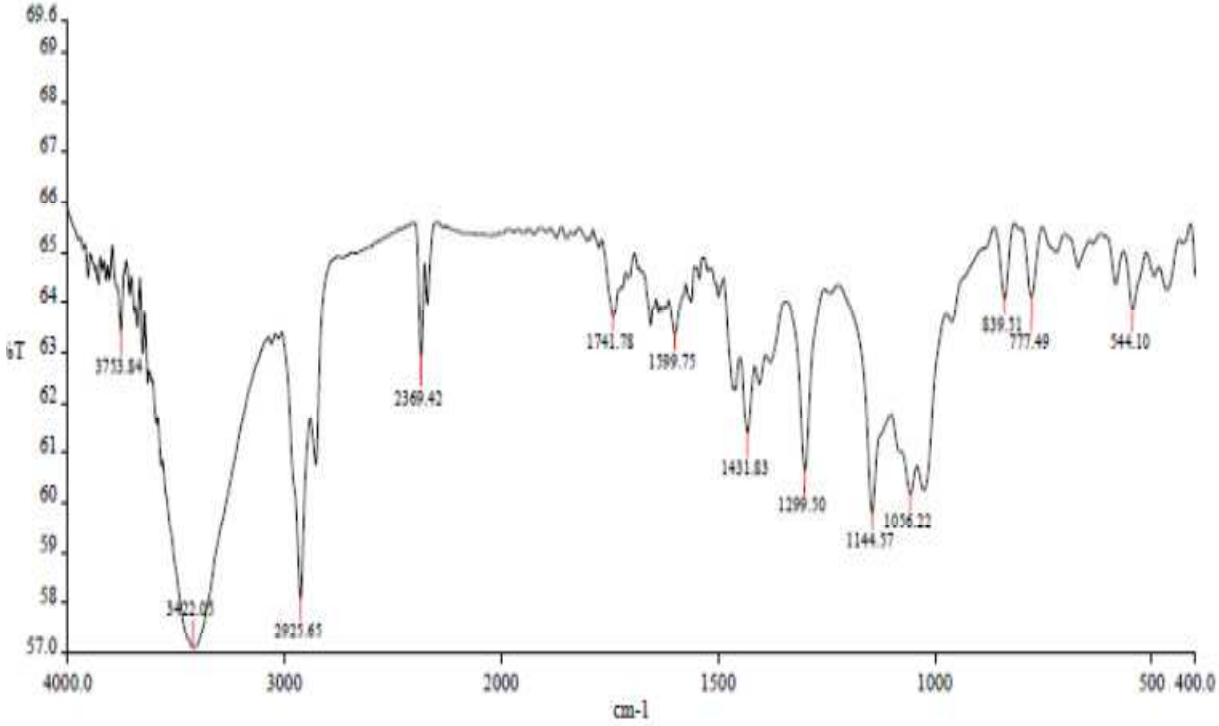


Figure 15d FT-IR spectra are as follows (g) Span 20 + Drug + Cholesterol, (h) Span 40 + Drug + Cholesterol

15(i) SPAN 60 +DRUG + CHOLESTEROL



15(j) SPAN 80 + DRUG + CHOLESTEROL

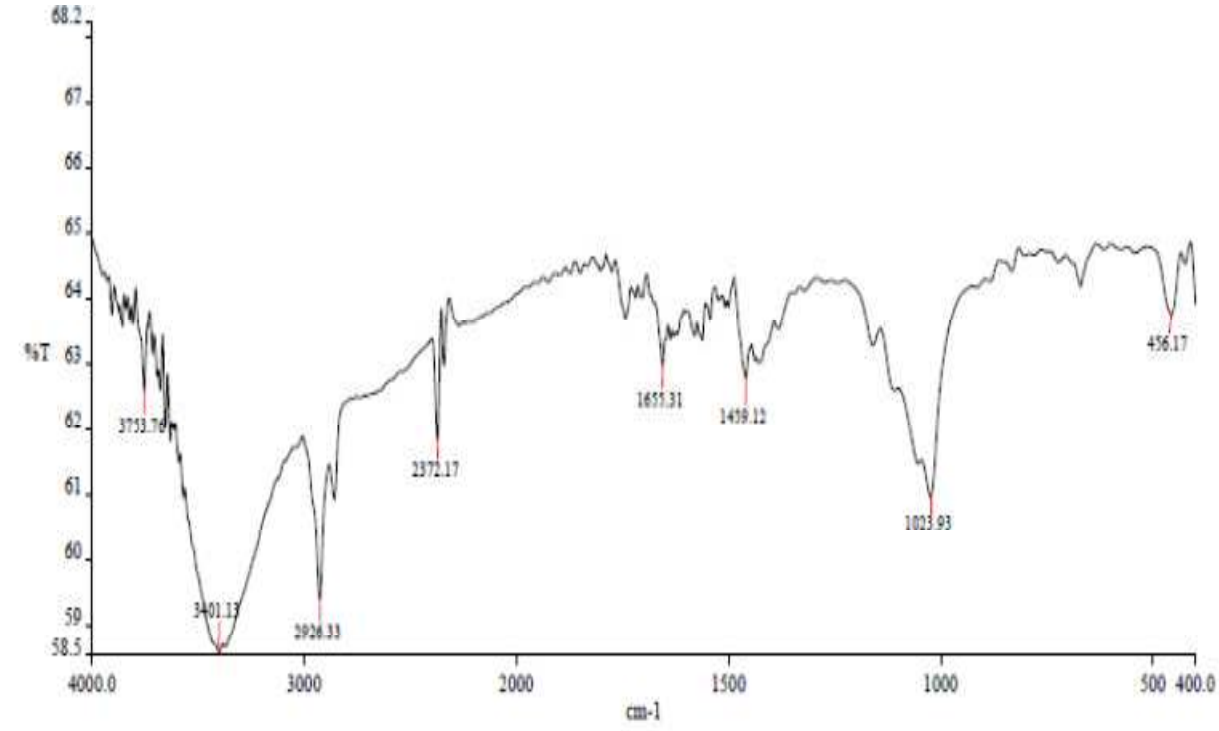
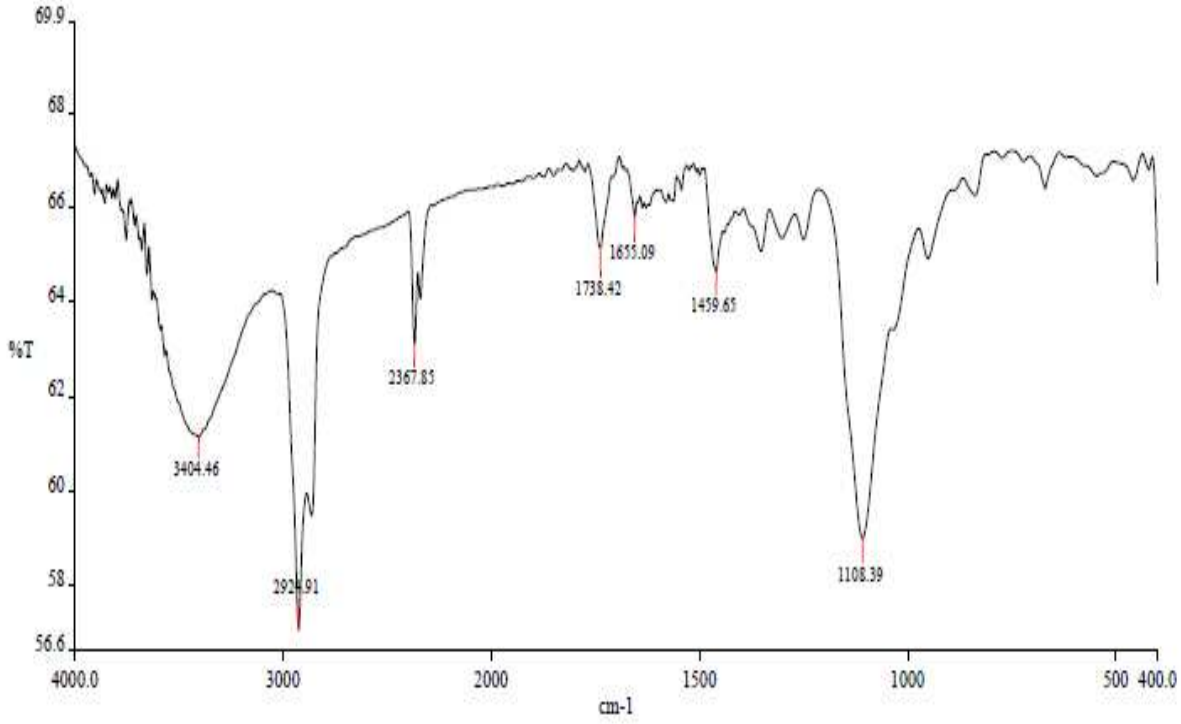


Figure 15e FT-IR spectra are as follows (i) Span 60 + Drug + Cholesterol, (j) Span80 + Drug + Cholesterol

15(k) TWEEN 60 + DRUG + CHOLESTEROL



15(l) TWEEN 80 + DRUG + CHOLESTEROL

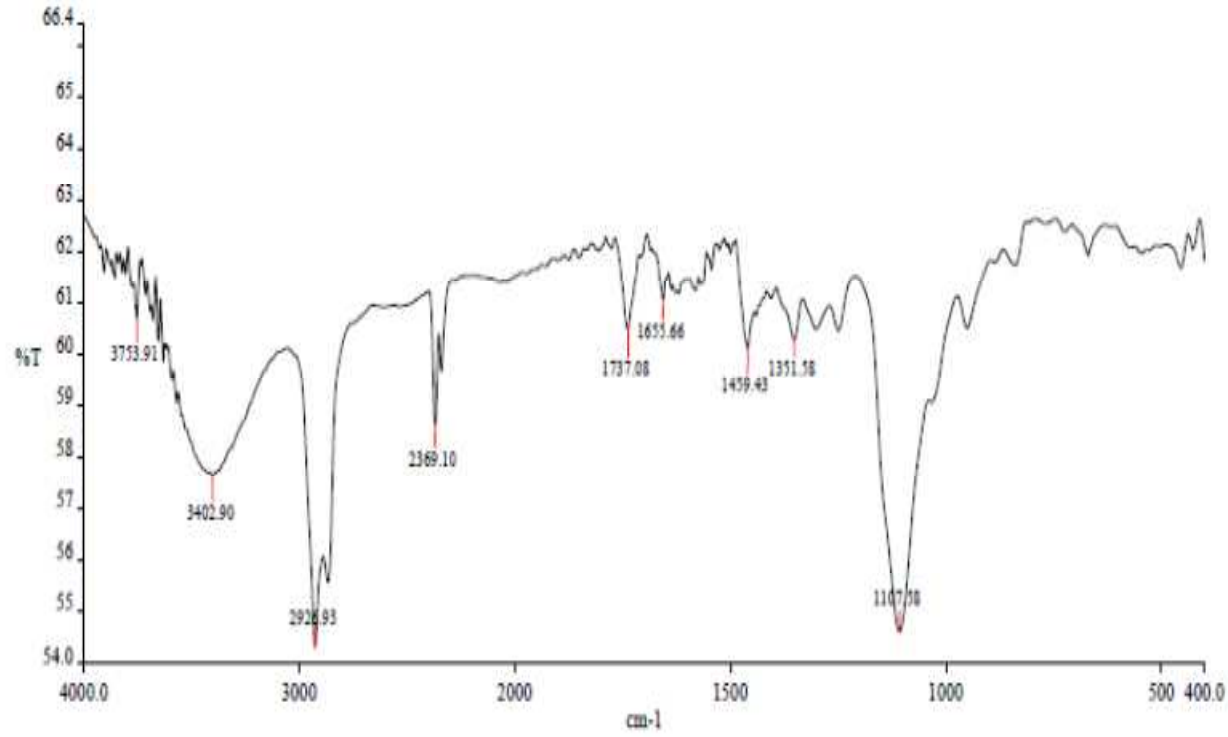
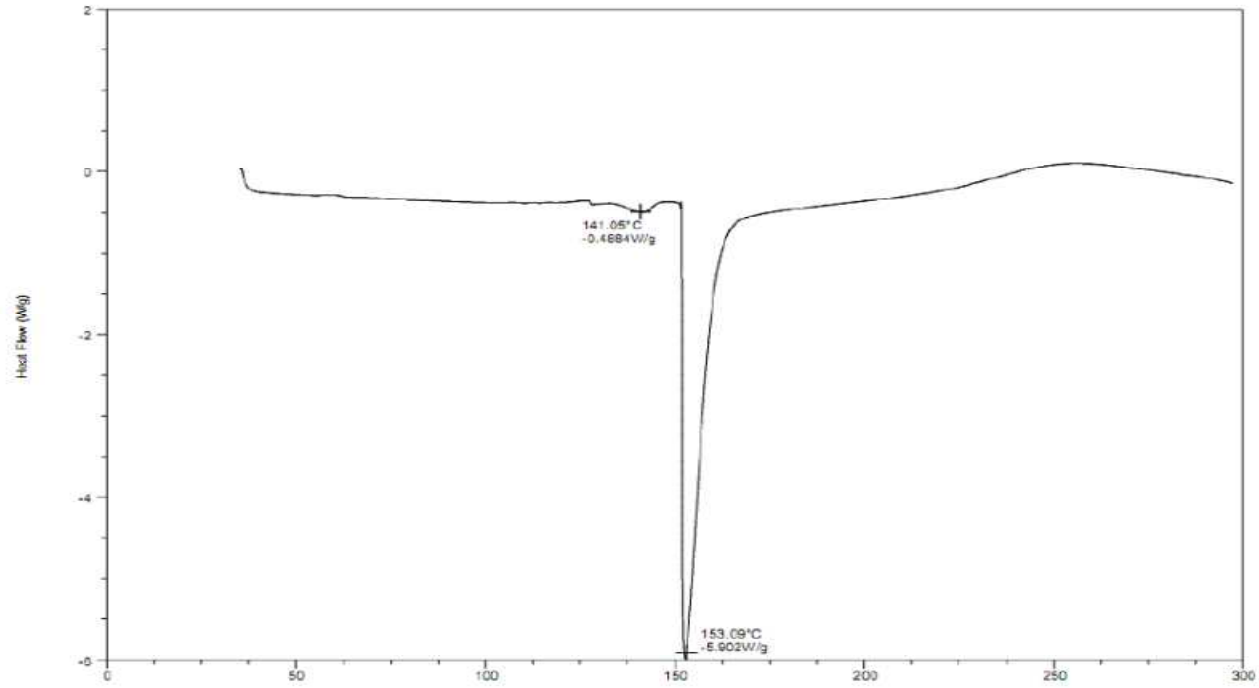


Figure 15f FT-IR spectra are as follows (k) Tween 60 + Drug + Cholesterol, (l) Tween80 + Drug + Cholesterol

16(a) ETORICOXIB



16(b) CHOLESTEROL

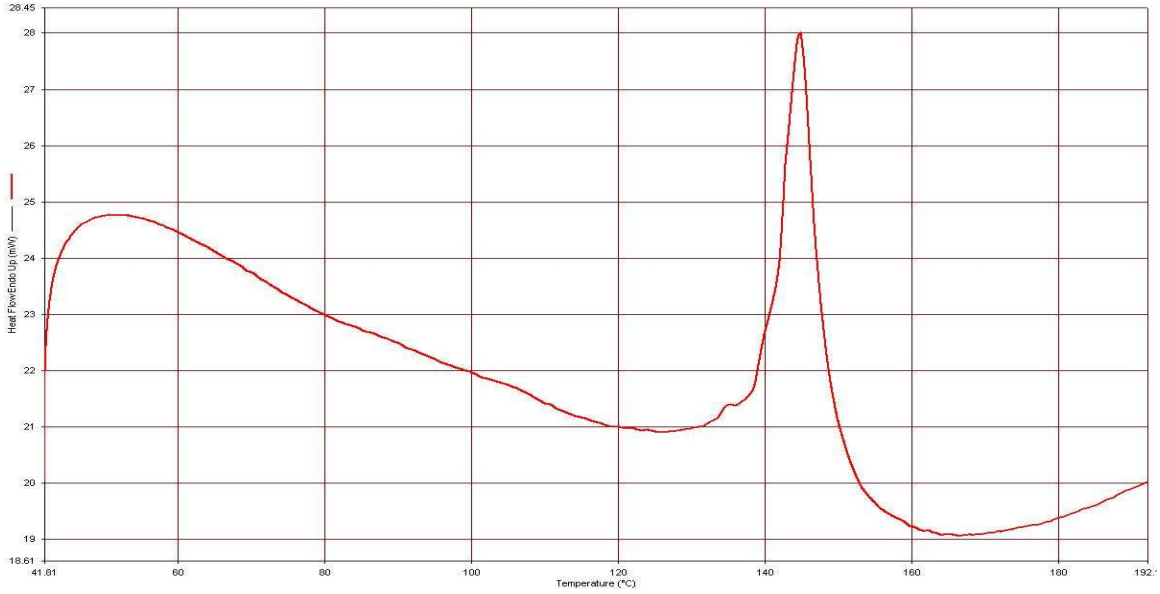
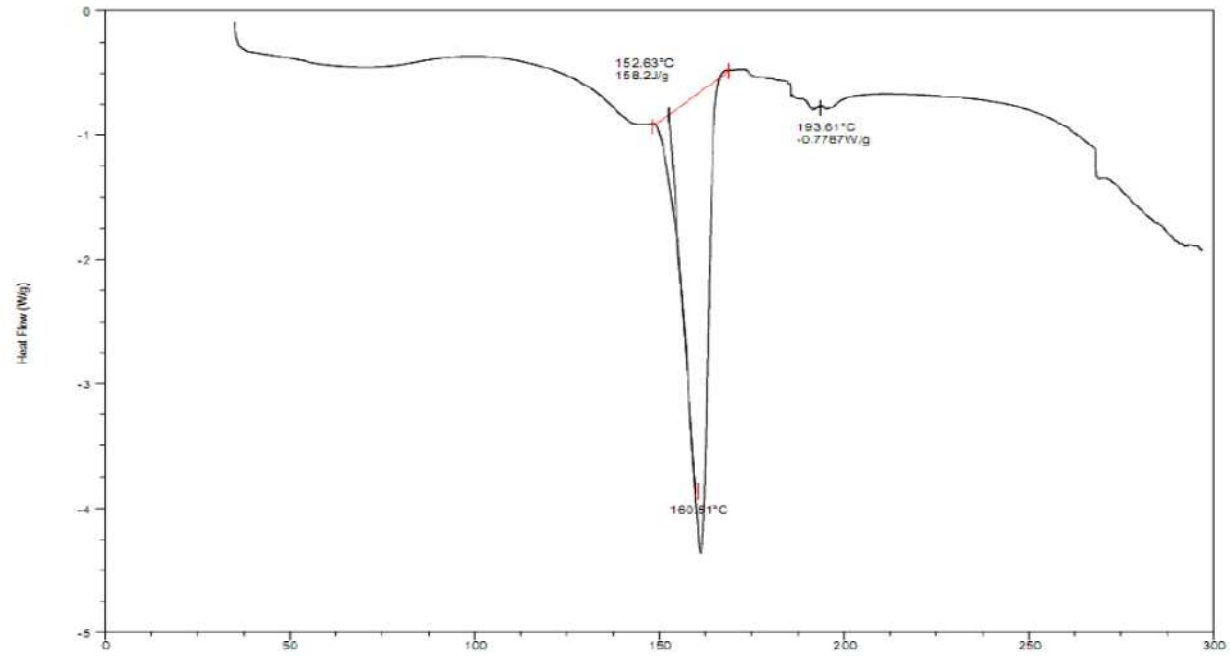


Figure 16a DSC Thermogram is as follows-(a) Etoricoxib (b) Cholesterol

16(c) SPAN 20



16(d) SPAN 40

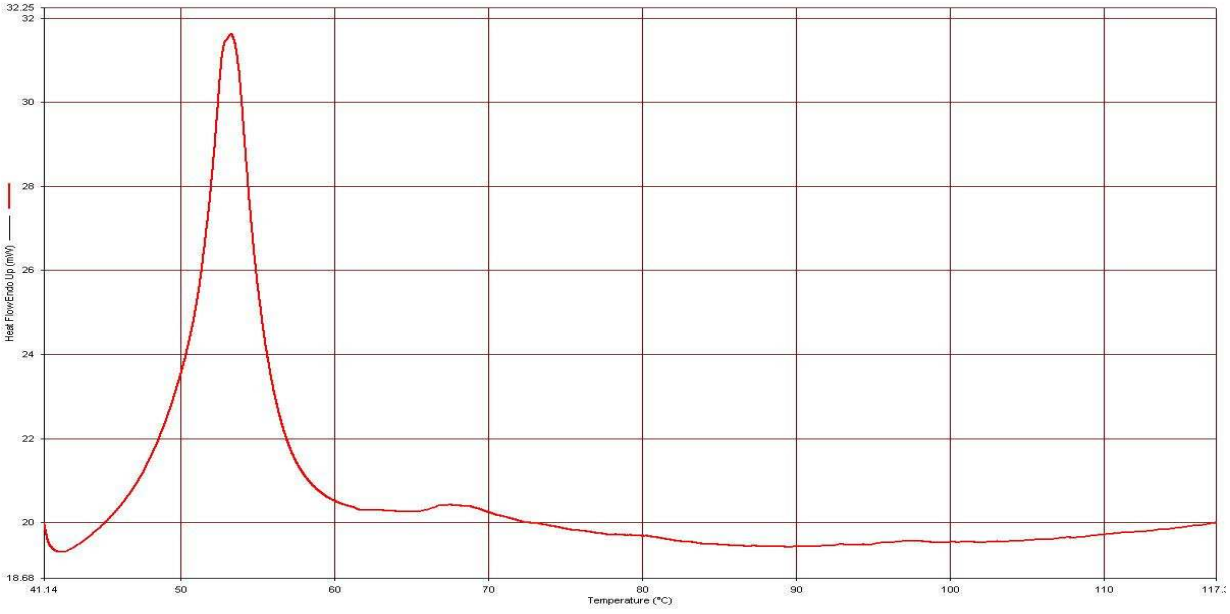
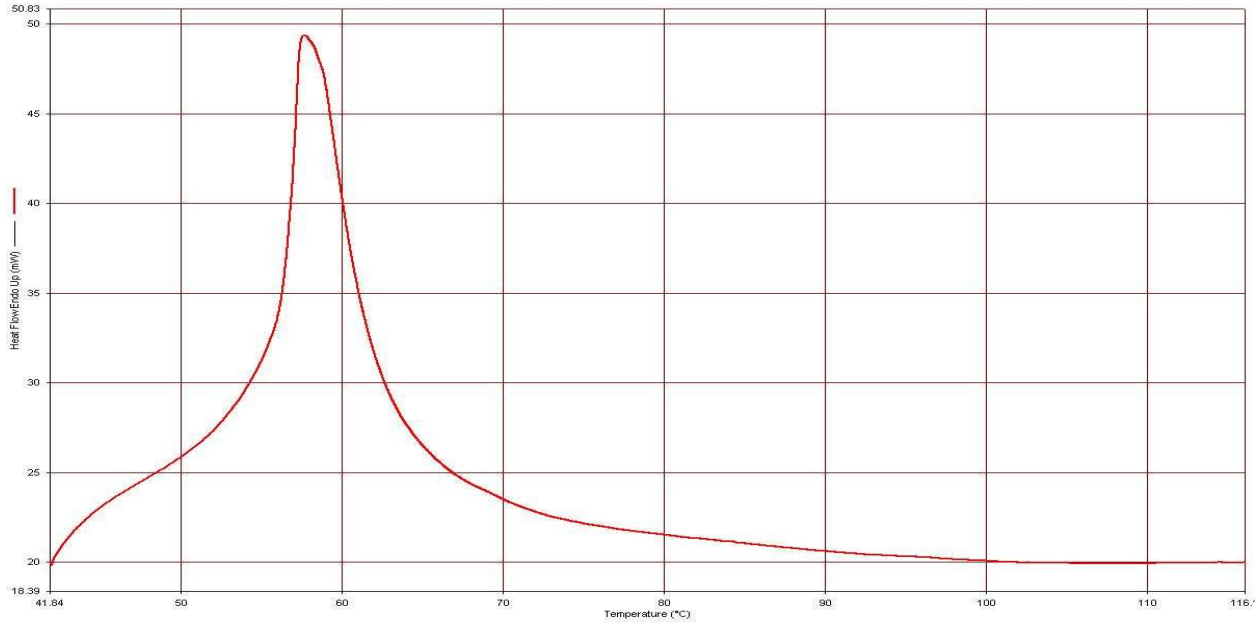


Figure 16b DSC Thermogram is as follows-(c) Span20 (d) Span40

16(e) SPAN 60



16(f) TWEEN 60

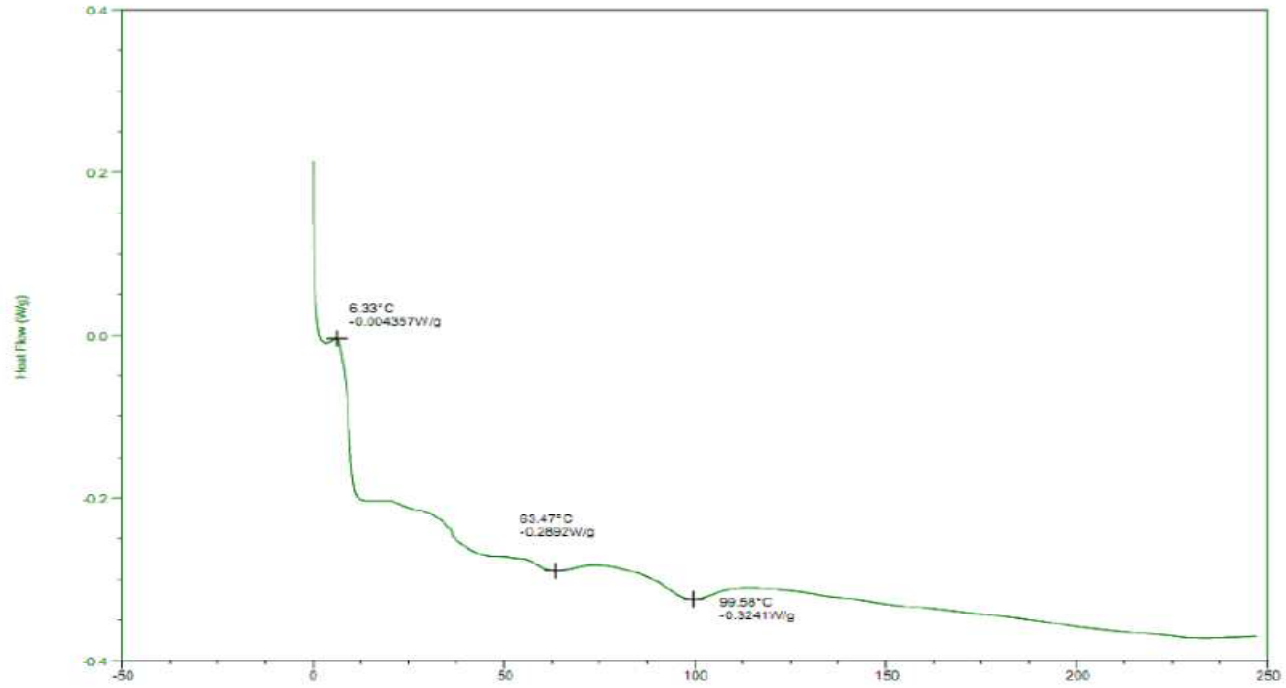
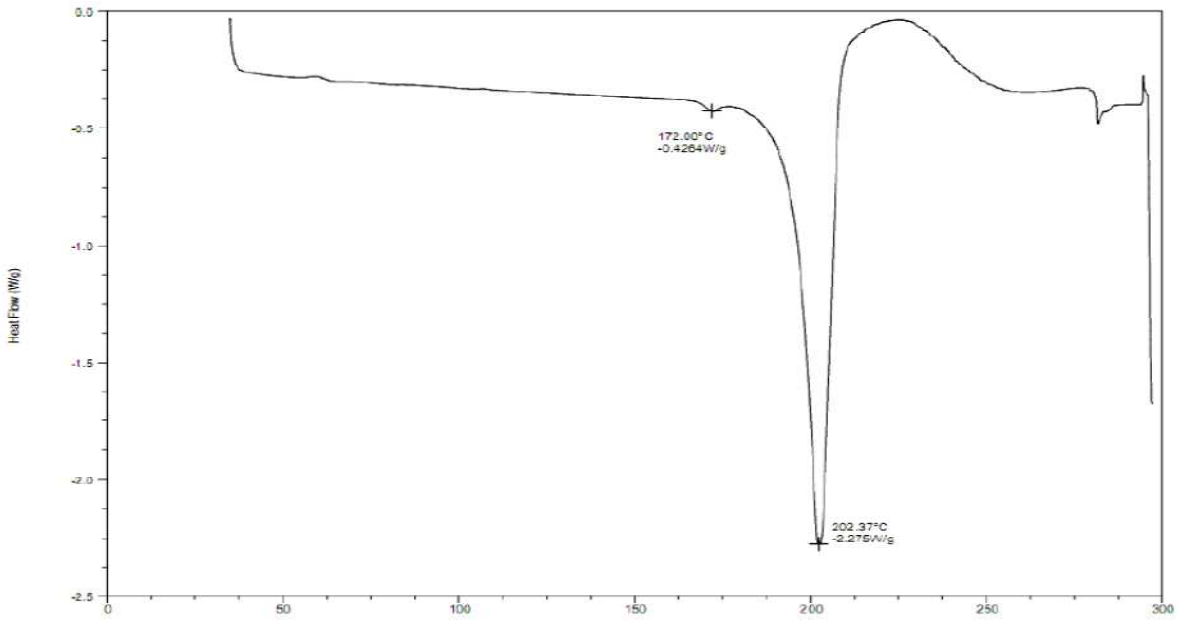


Figure 16c DSC Thermogram is as follows-(e) Span60 (f)Tween60

16(g) BRIJ-52



16(h) SPAN 20+ DRUG+CHOLESTEROL

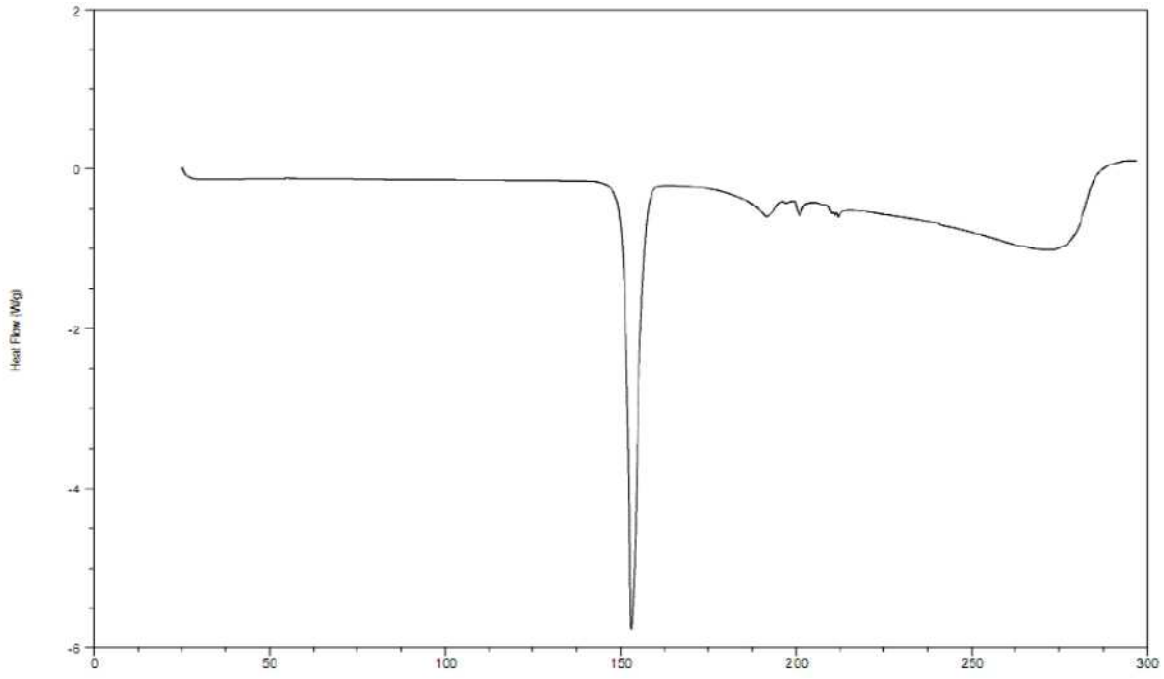
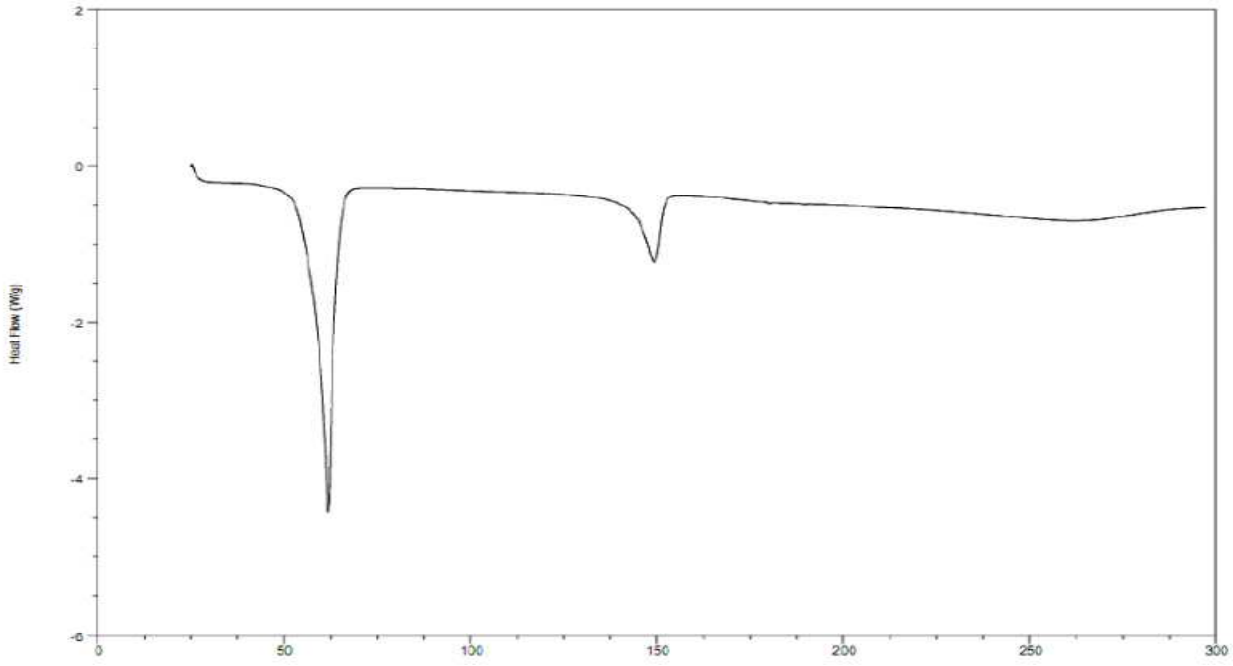


Figure 16d DSC Thermogram is as follows-(g) Brij-52(h) Span 20+ Drug +Cholesterol

16(i) SPAN 40 +DRUG+CHOLESTEROL



16(j) SPAN 60+DRUG+CHOLESTEROL

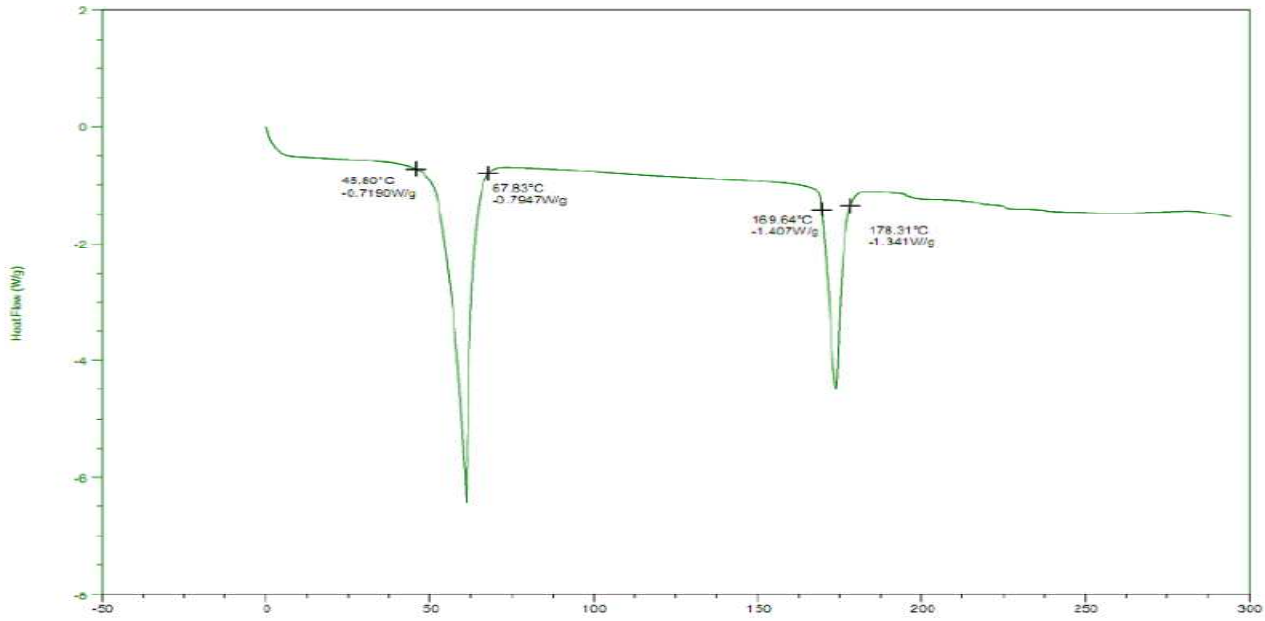


Figure 16 e DSC Thermogram are as follows-(I) Span 40 +Drug+ Cholesterol (J) Span 60+Drug+Cholesterol

16(k) BRIJ-52+ DRUG+ CHOLESTEROL

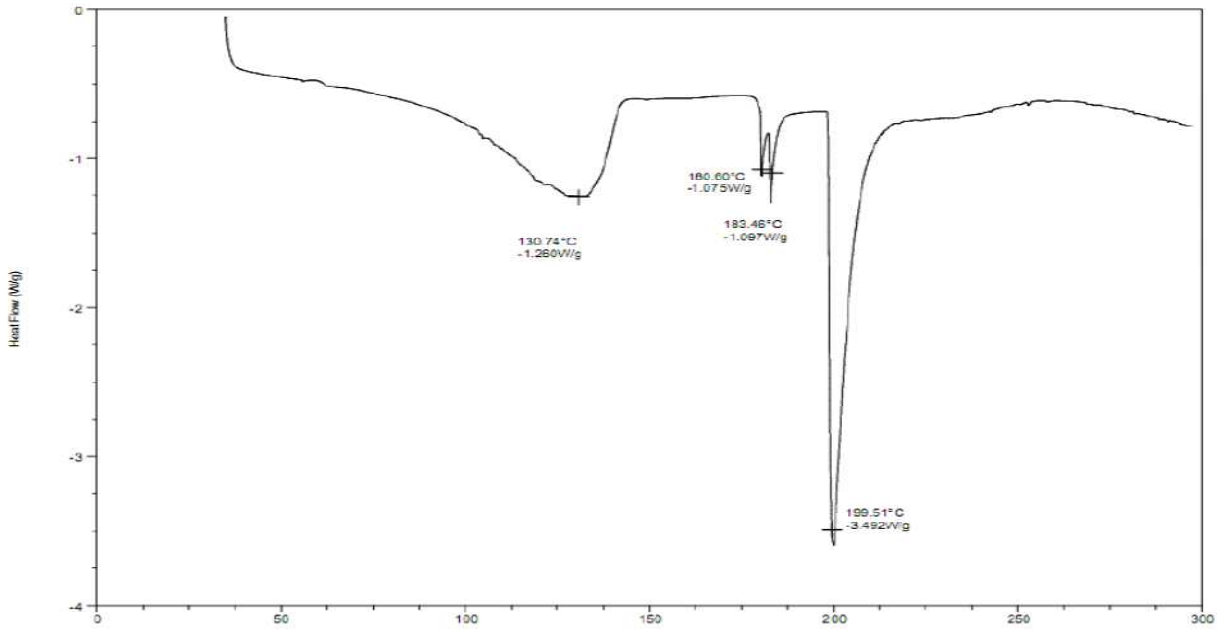


Figure 16f DSC Thermogram are as follows- (K) Brij-52+ Drug+ Cholesterol

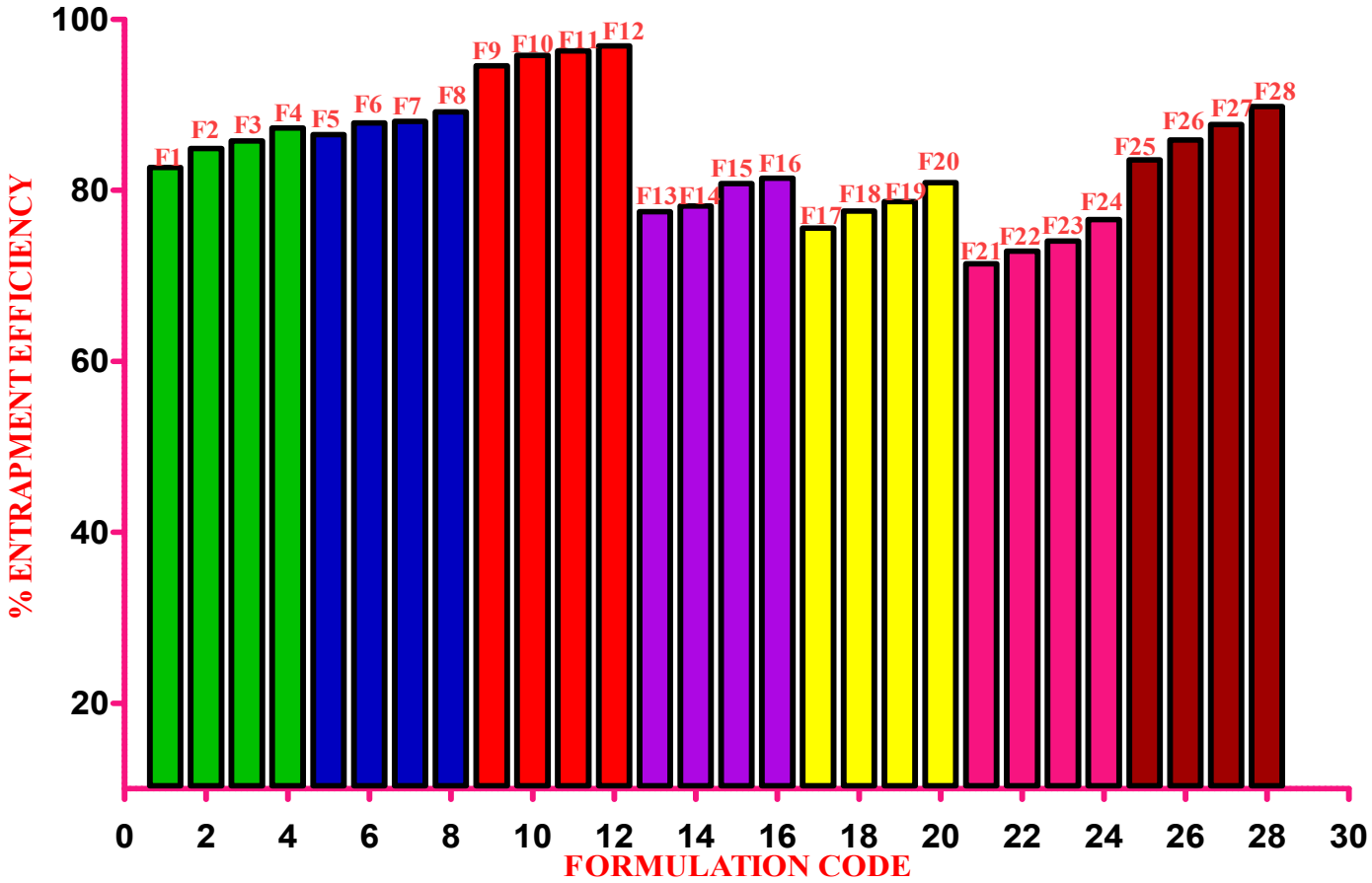


Figure 17 Comparison of Percentage Entrapment Efficiency of Different Surfactant in Different Ratios

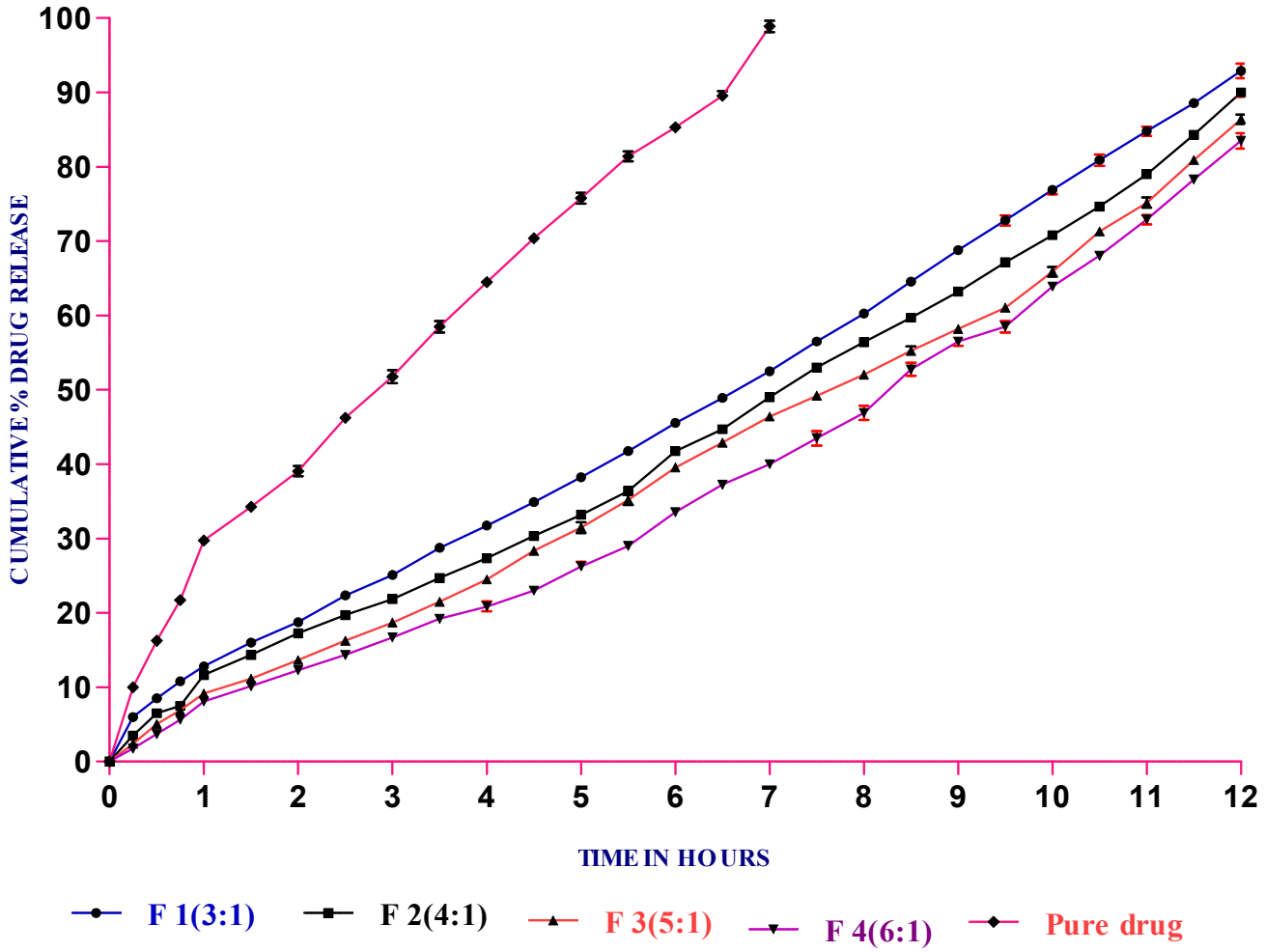


Figure 18a Comparison of *Invitro* Release of Niosomes Containing Span20 in Different Ratio

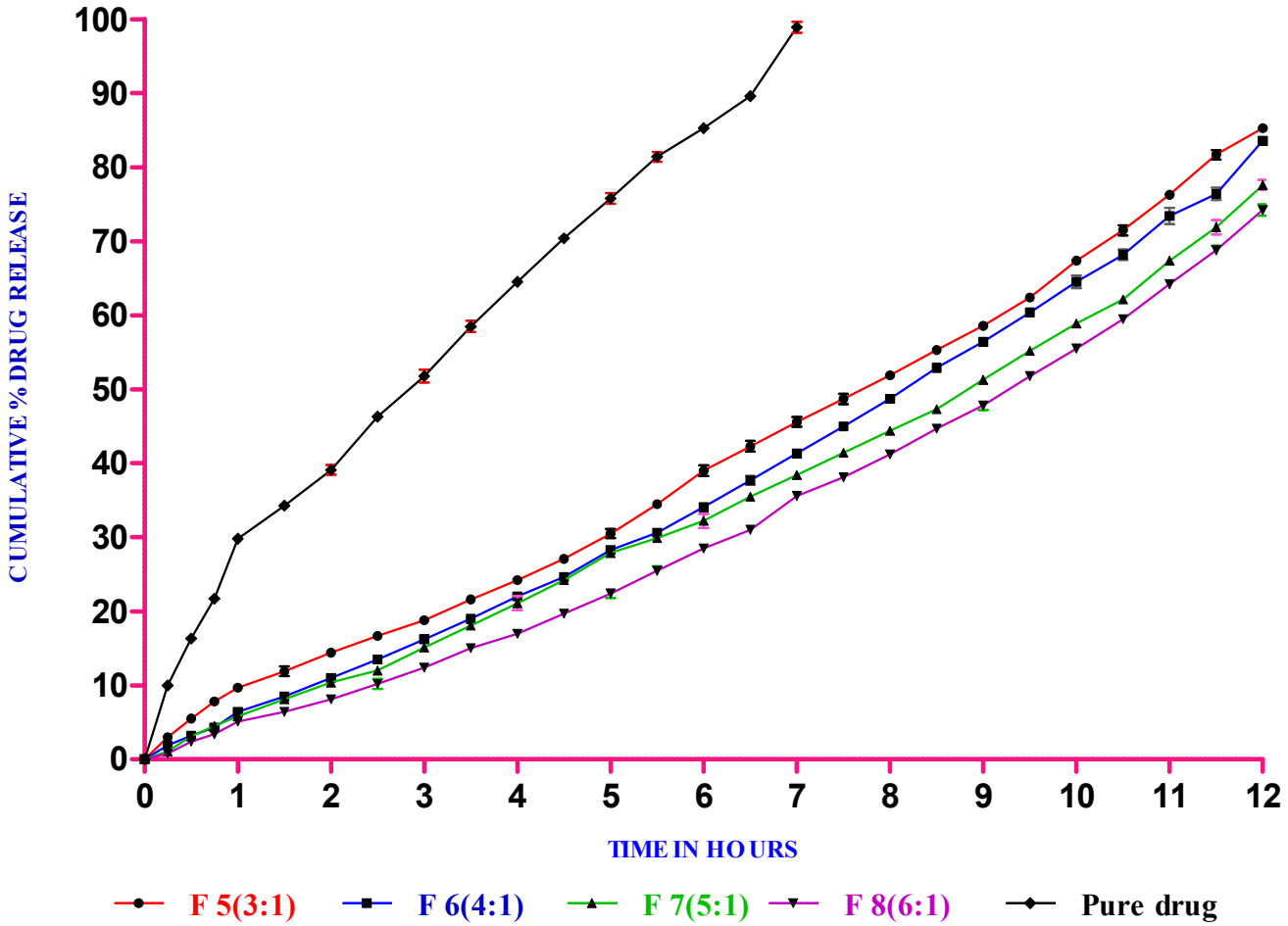


Figure 18b Comparison of *invitro* release of niosomes containing span40 in different ratio

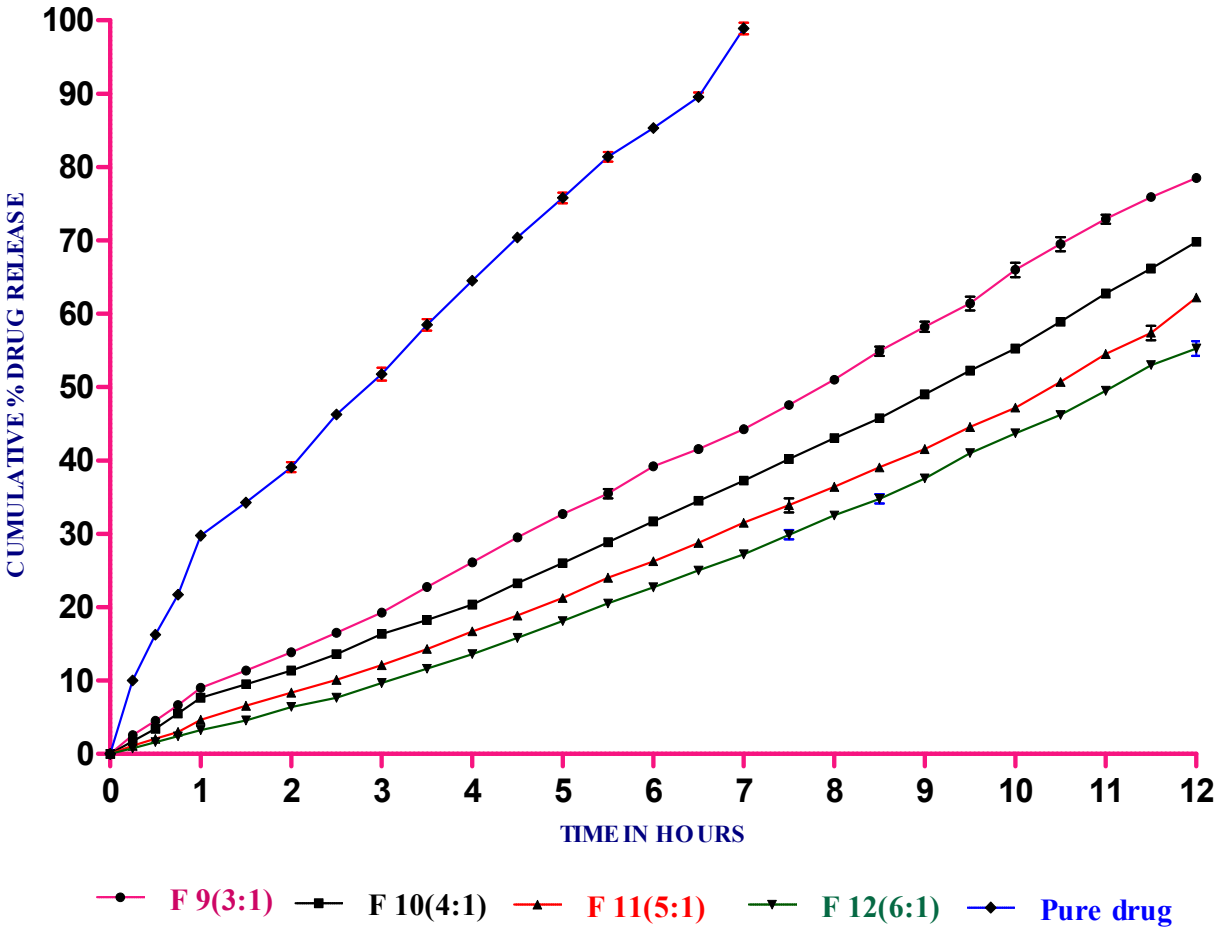


Figure 18 c Comparison of *Invitro* Release of Niosomes Containing Span60 In Different Ratio

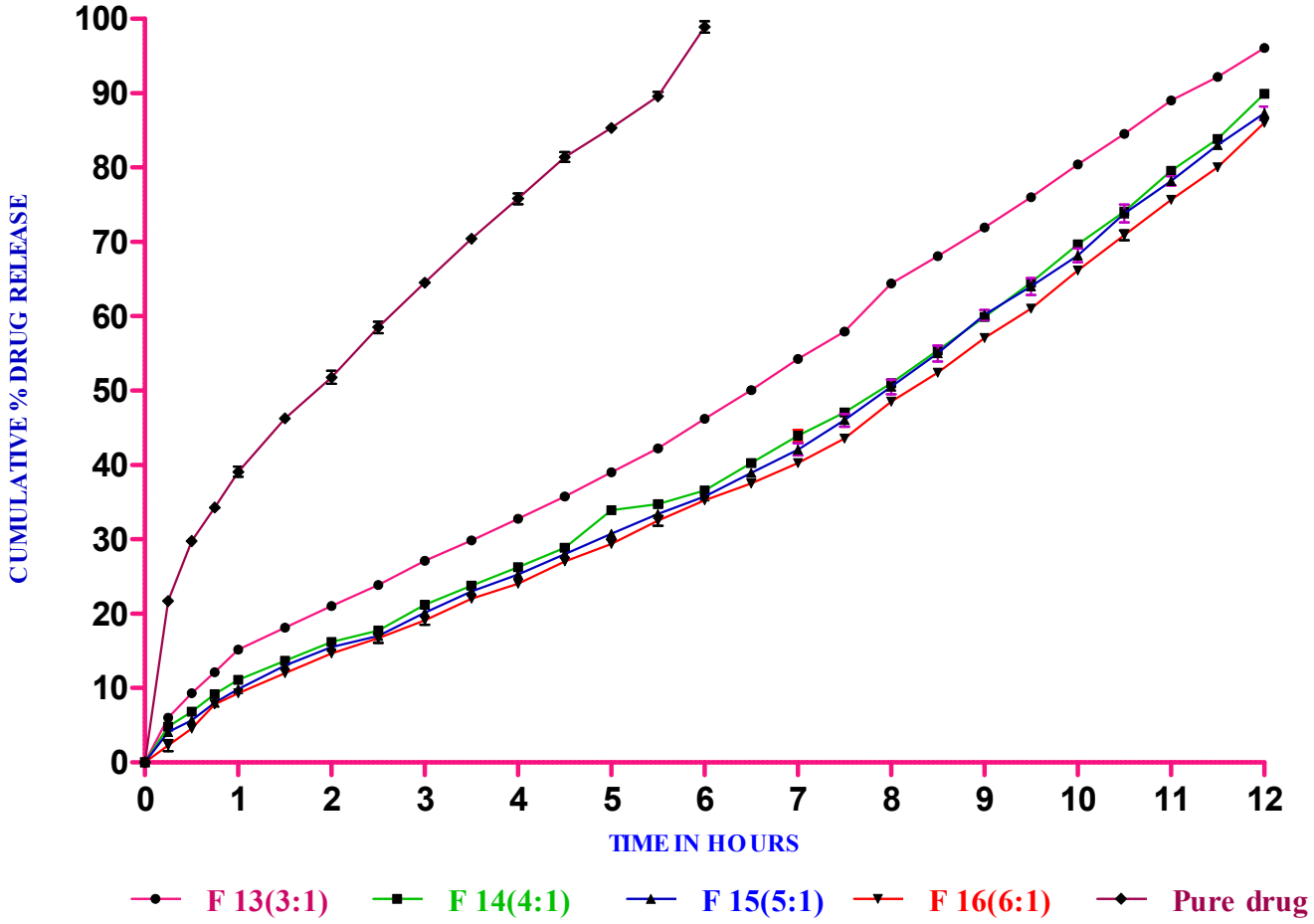


Figure18d Comparison of *Invitro* Release of Niosomes Containing Span80 in Different Ratio

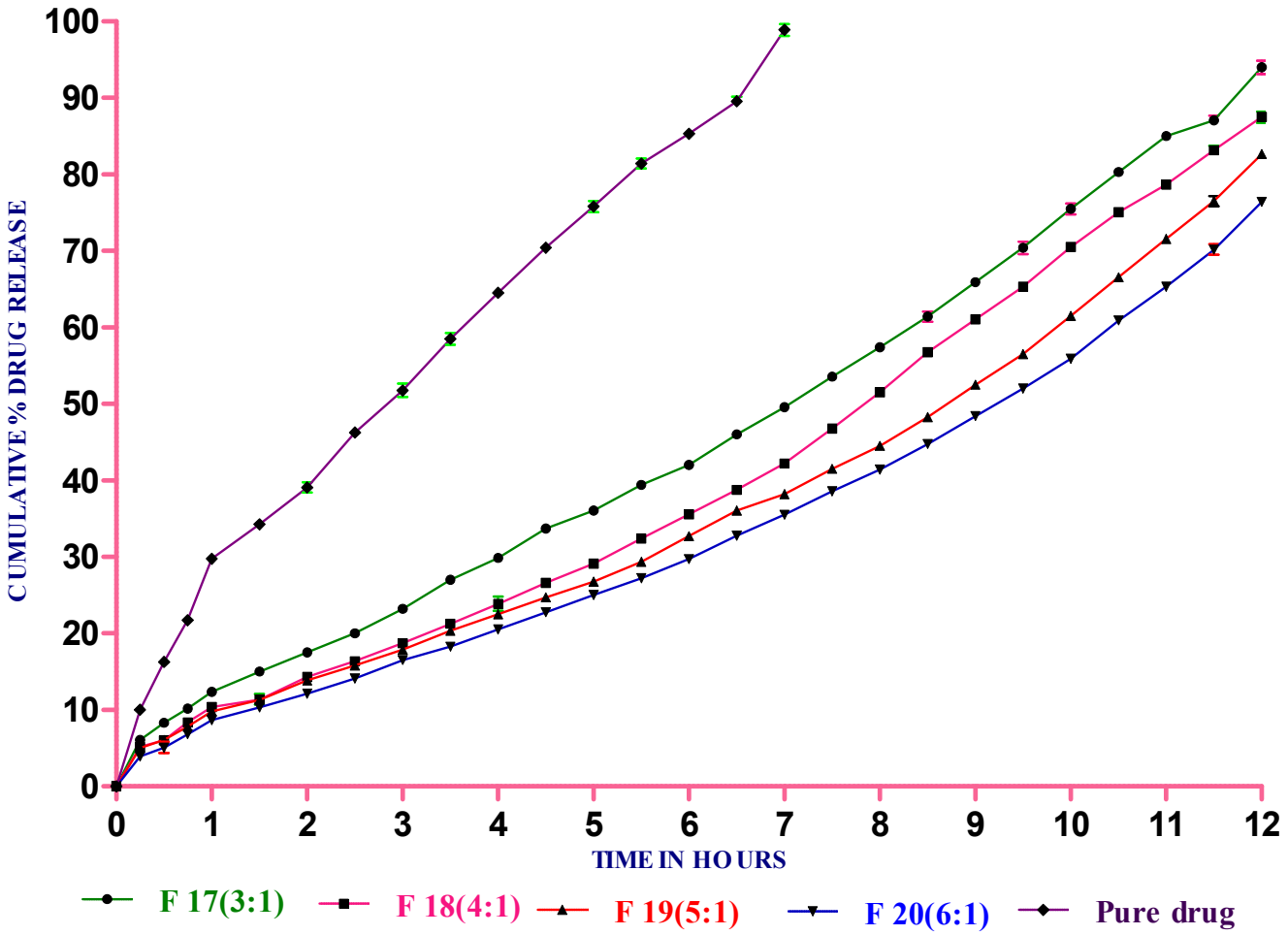


Figure 18 e Comparison of *Invitro* Release of Niosomes Containing Tween60 in Different Ratio

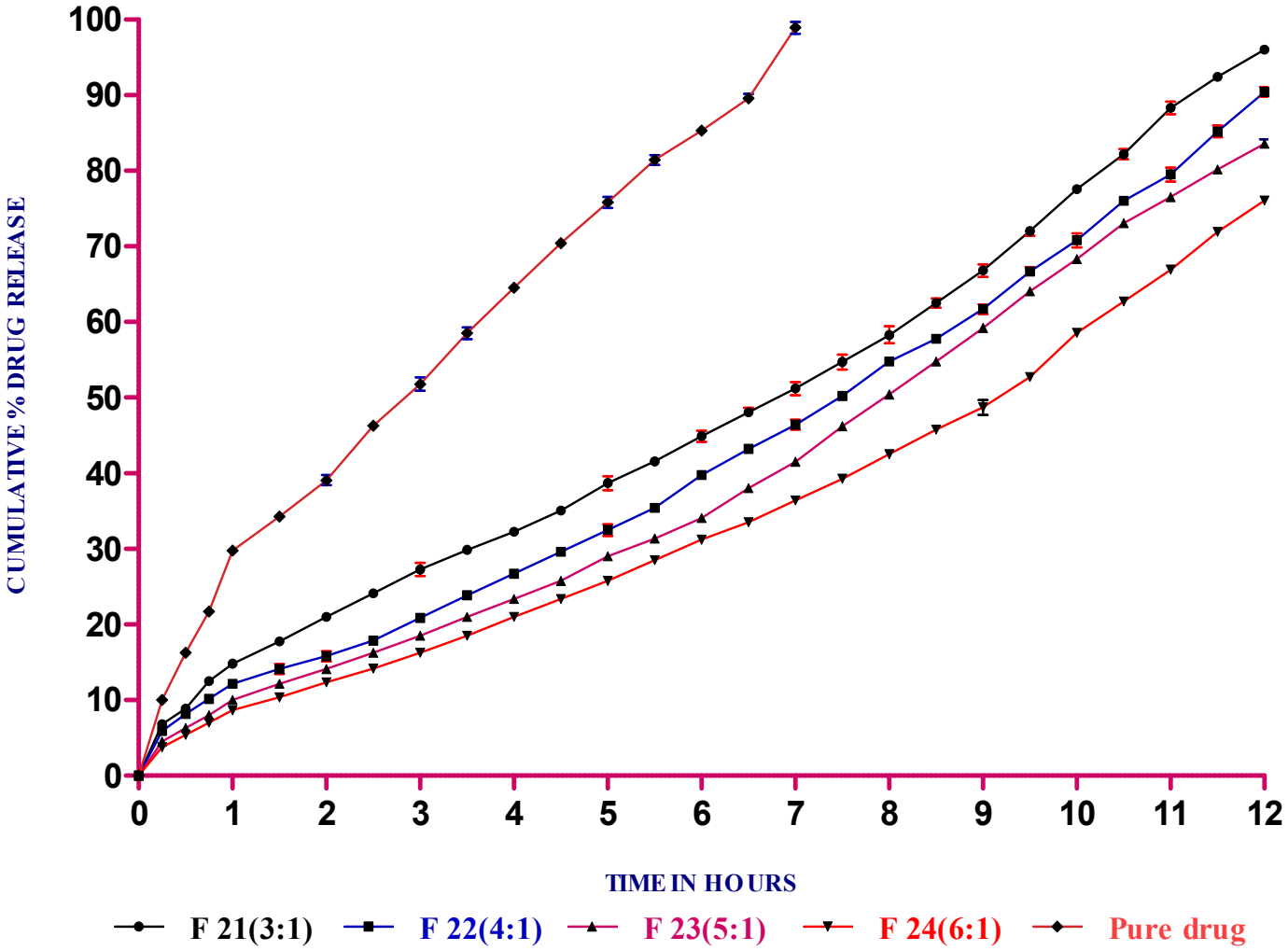


Figure18f Comparison of *Invitro* Release of Niosomes Containing Tween80 inDifferent Ratio

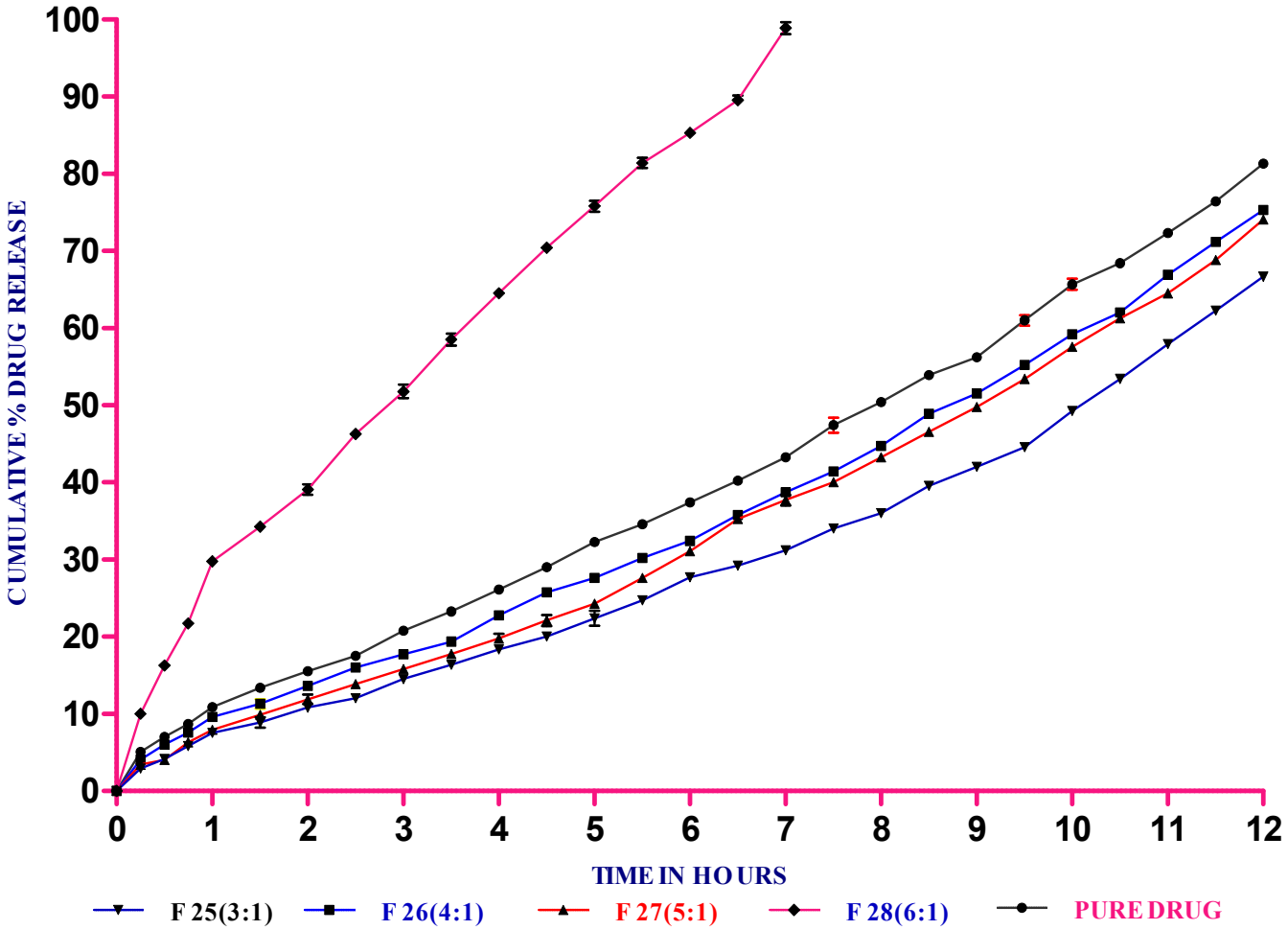
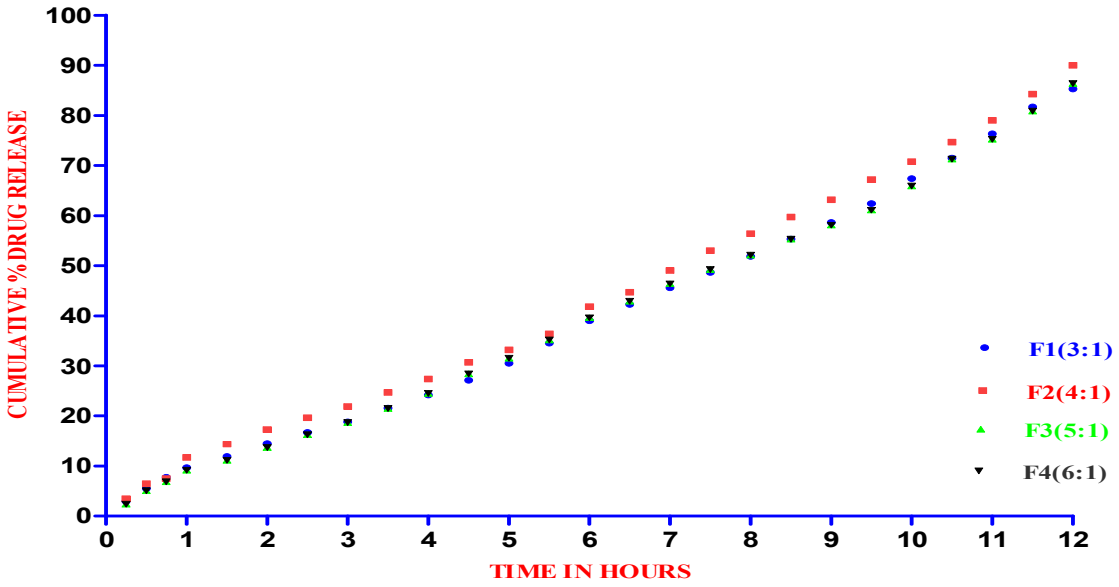
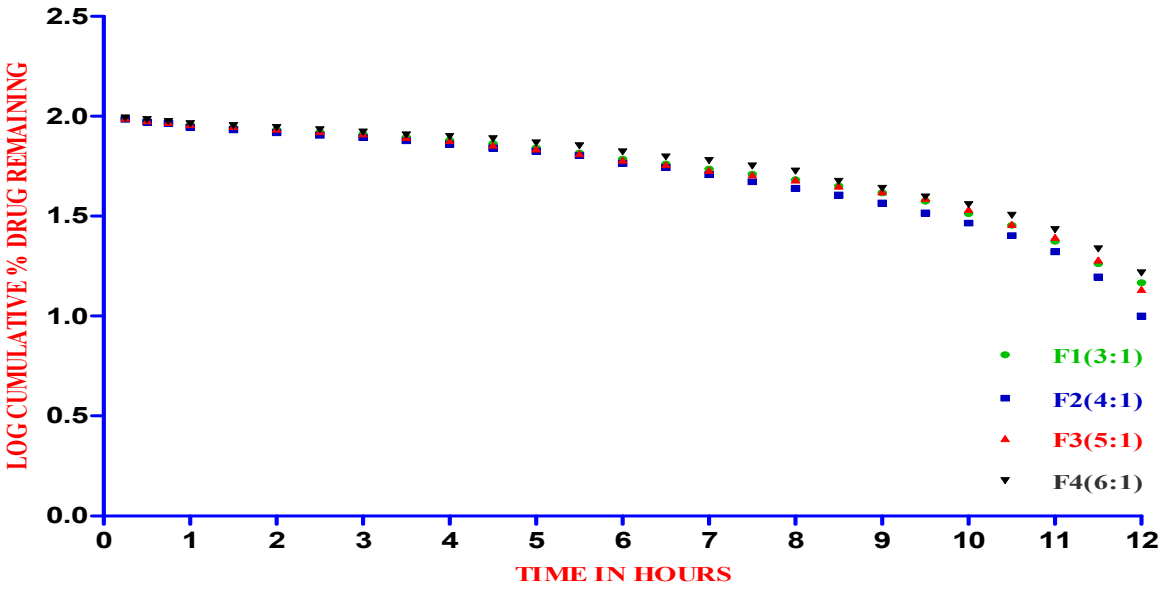


Figure 18g Comparison of *Invitro* Release of Niosomes Containing Brij-52 in Different Ratio

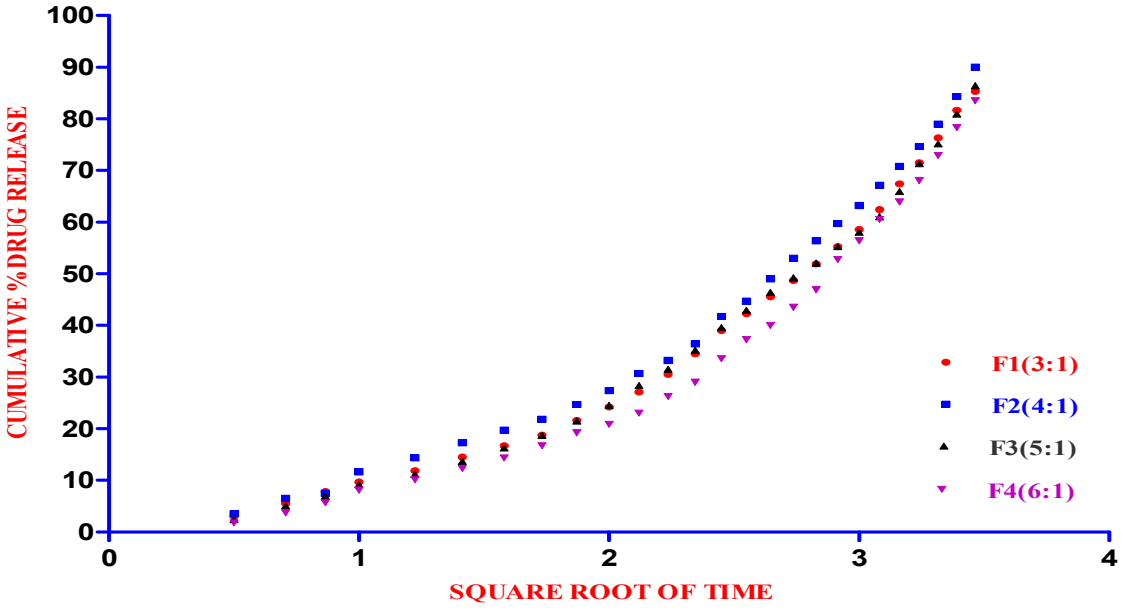
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF SPAN-20 AT DIFFERENT RATIO



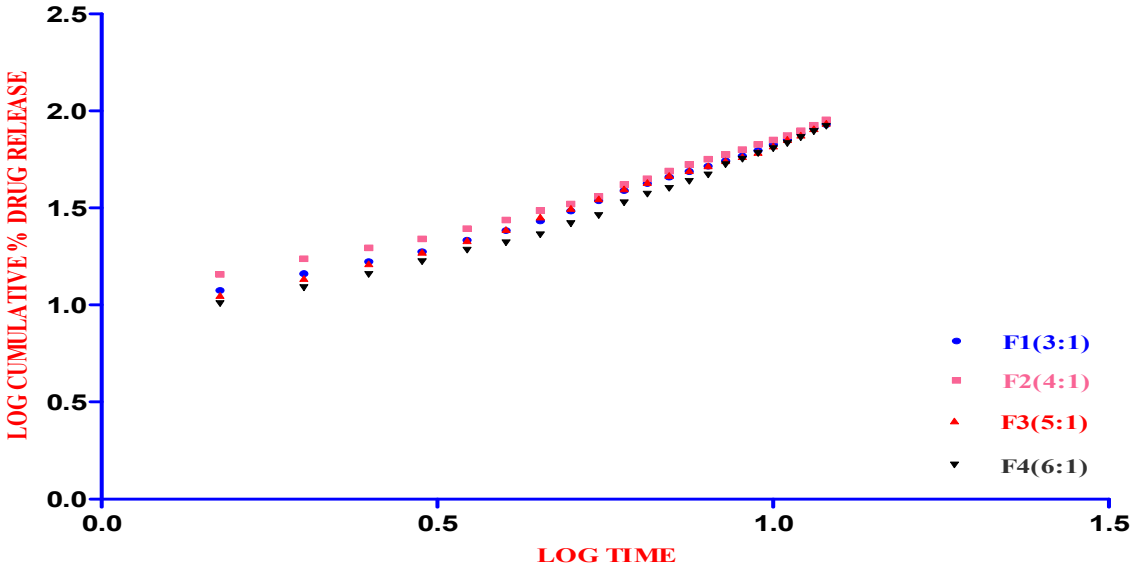
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF SPAN-20 AT DIFFERENT RATIO



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF SPAN-20 AT DIFFERENT RATIO



COMPARISION OF INVITRO KORSMEYER&PEPPAS MODEL RELEASE KINETICS OF SPAN-20 AT DIFFERENT RATIO



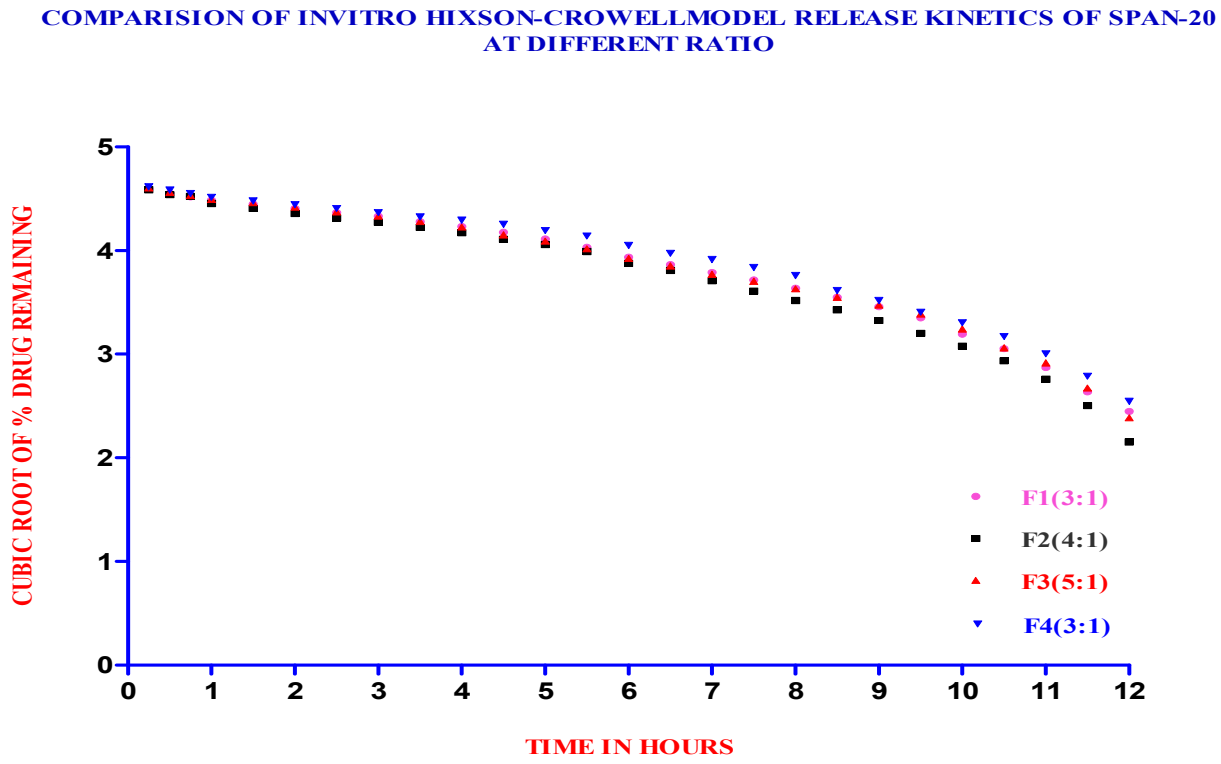
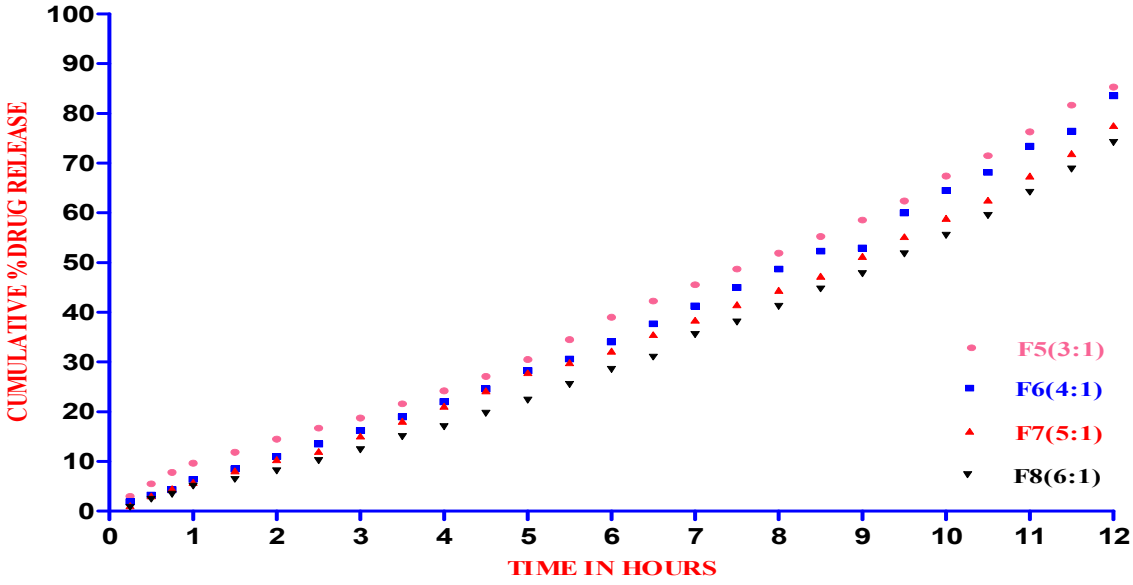
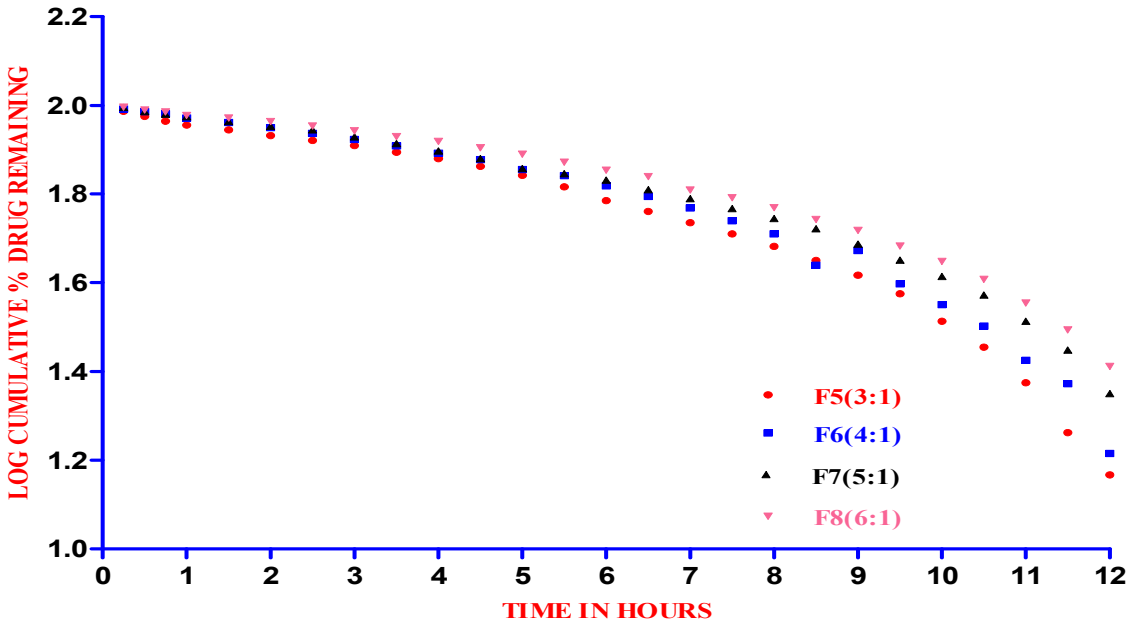


Figure 19a ***invitro*** **Release Kinetics of Niosomal Formulation Containing Span20 in Different Ratio**

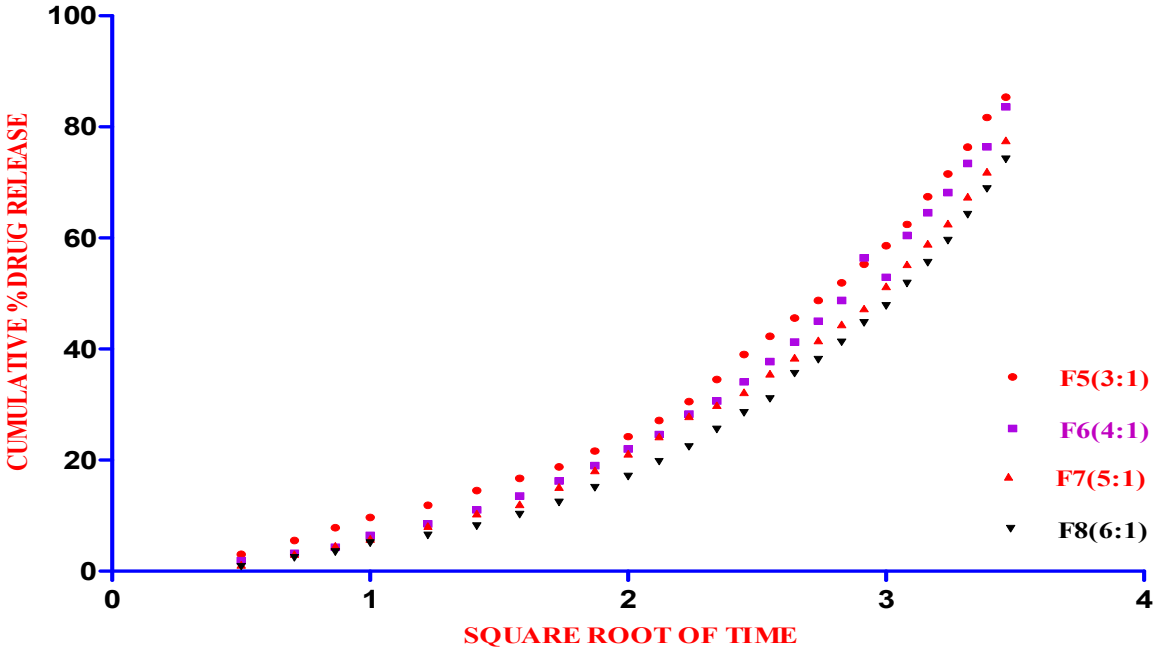
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF SPAN-40 AT DIFFERENT RATIO



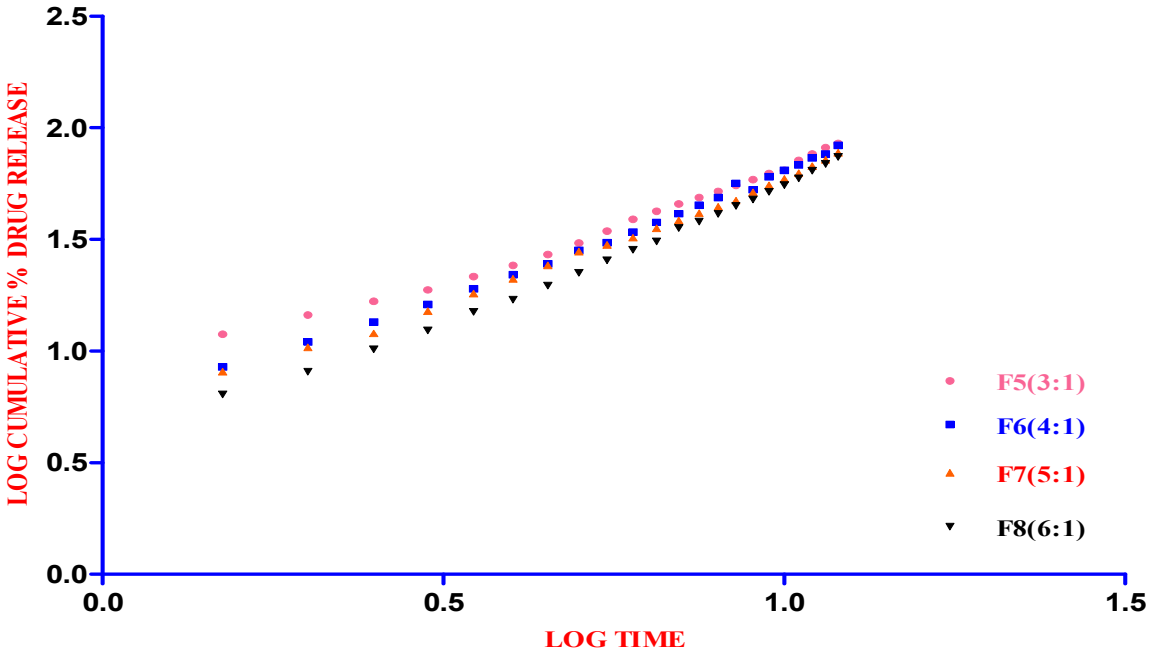
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF SPAN-40 AT DIFFERENT RATIO



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF SPAN-40 AT DIFFERENT RATIO



COMPARISION OF INVITRO KORSMEYER&PEPPAS MODEL RELEASE KINETICS OF SPAN-40 AT DIFFERENT RATIO



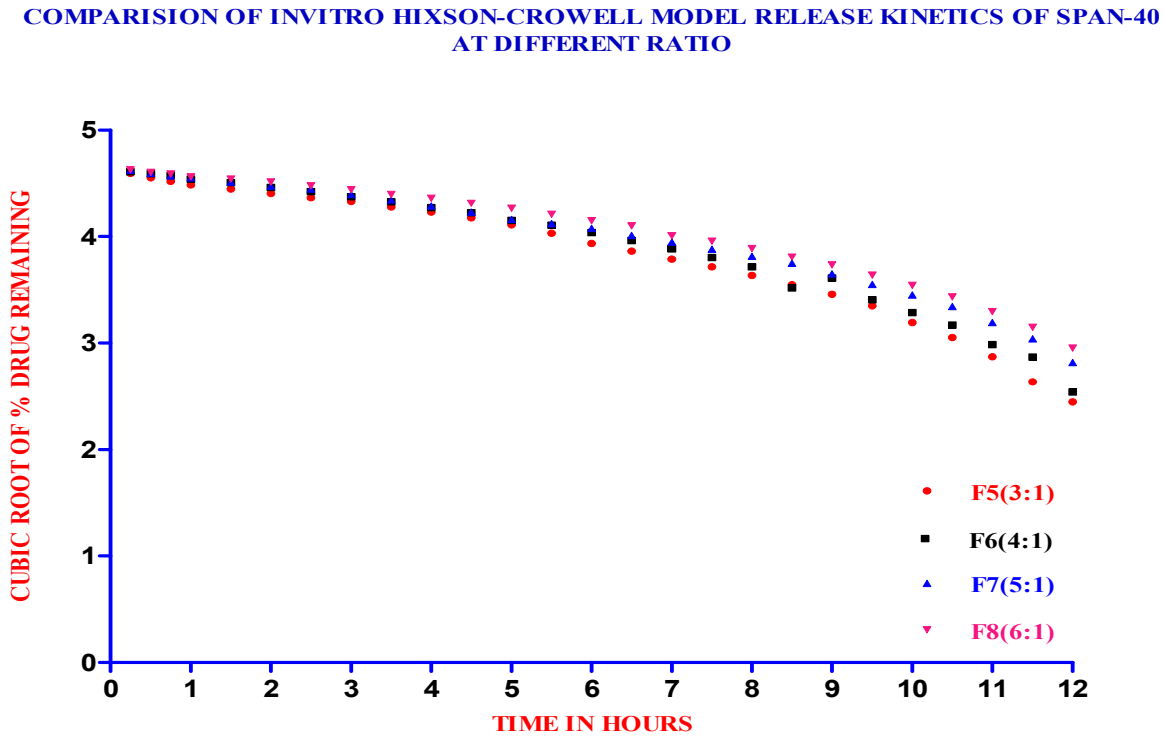
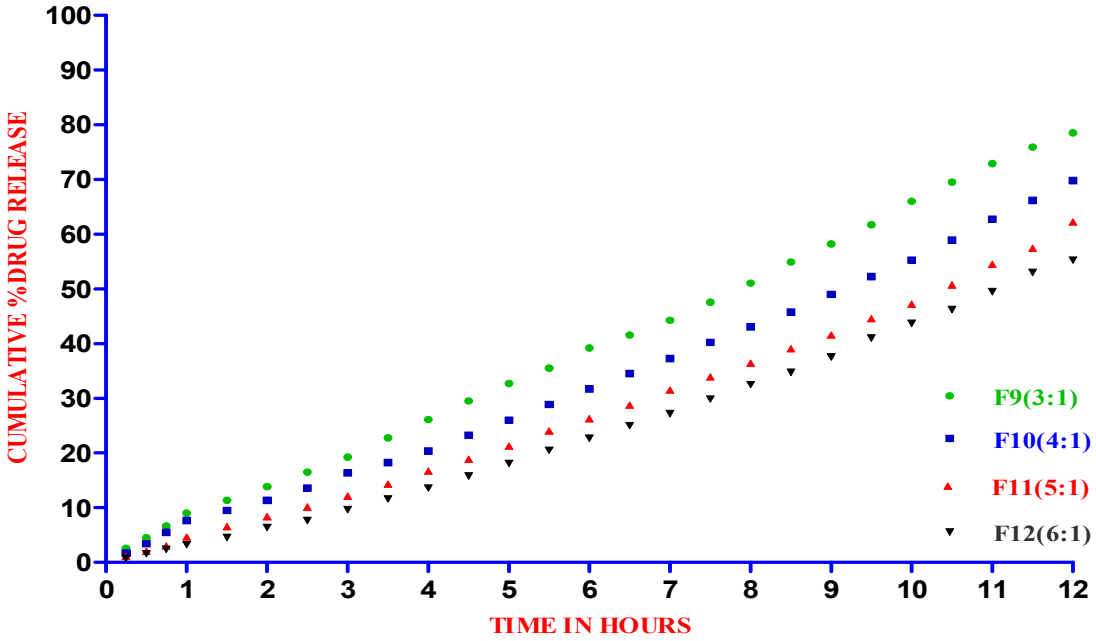
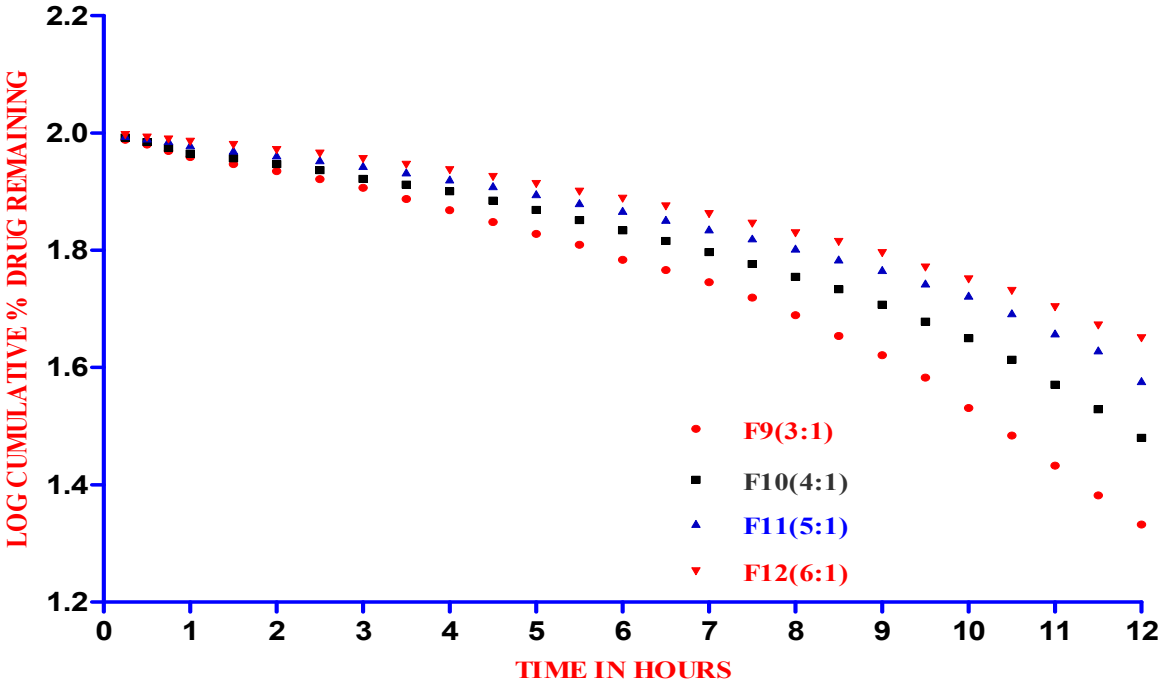


Figure 19b ***invitro*** **Release Kinetics of Niosomal Formulation Containing Span40 in Different Ratio**

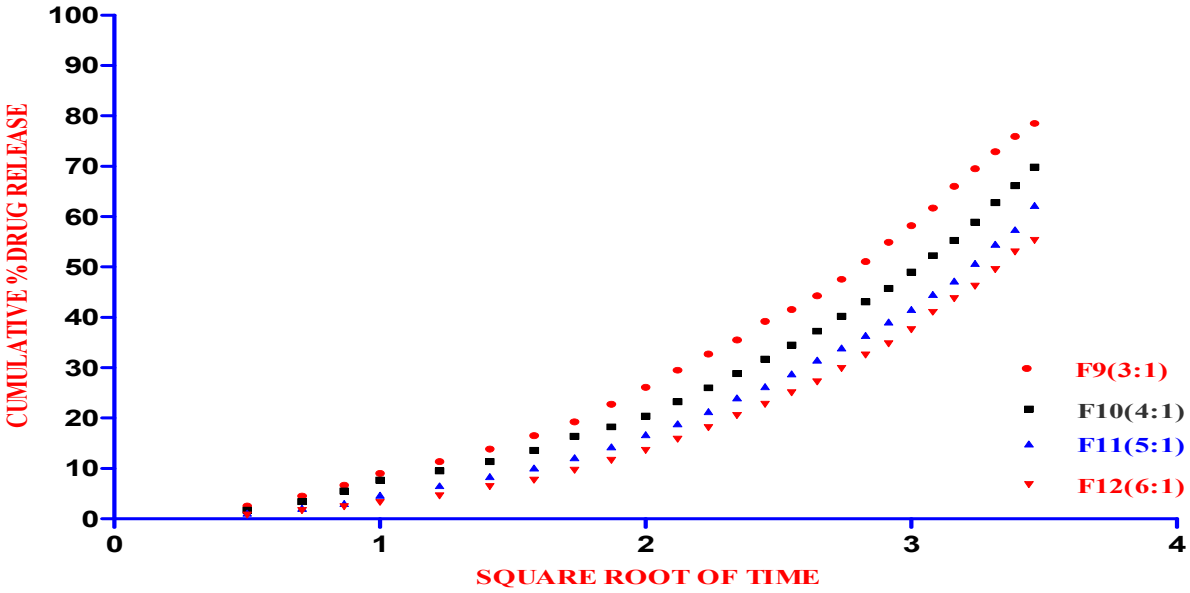
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF SPAN-60 AT DIFFERENT RATIO



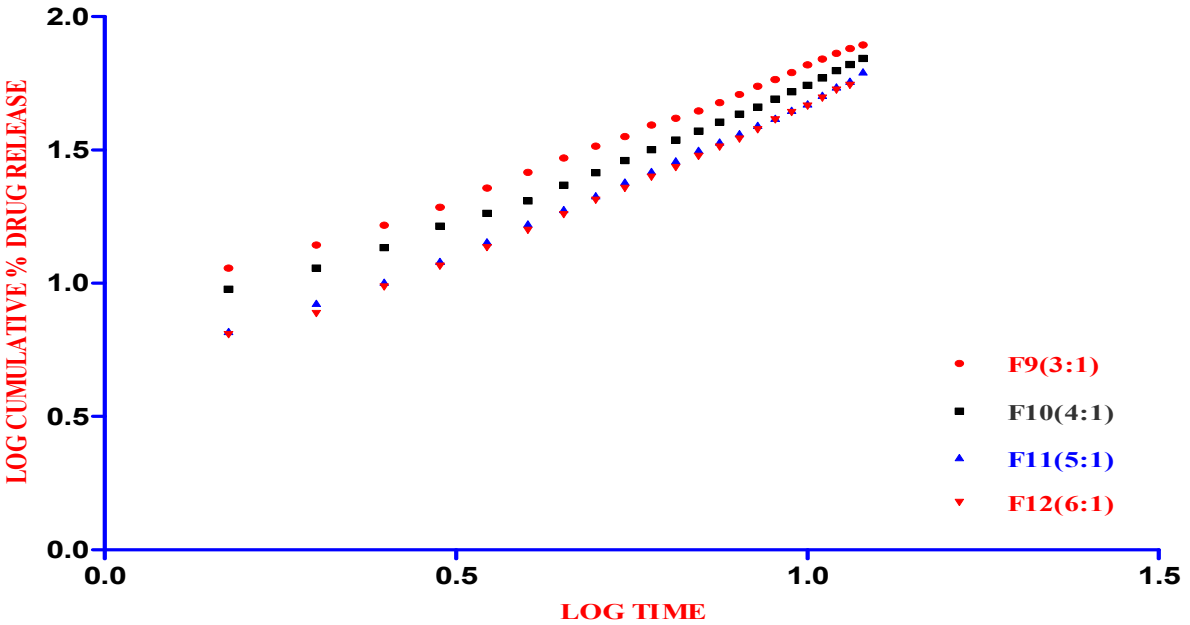
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF SPAN-60 AT DIFFERENT RATIO



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF SPAN-60 AT DIFFERENT RATIO



COMPARISION OF INVITRO KORSMEYER&PEPPAS MODEL RELEASE KINETICS OF SPAN-60 AT DIFFERENT RATIO



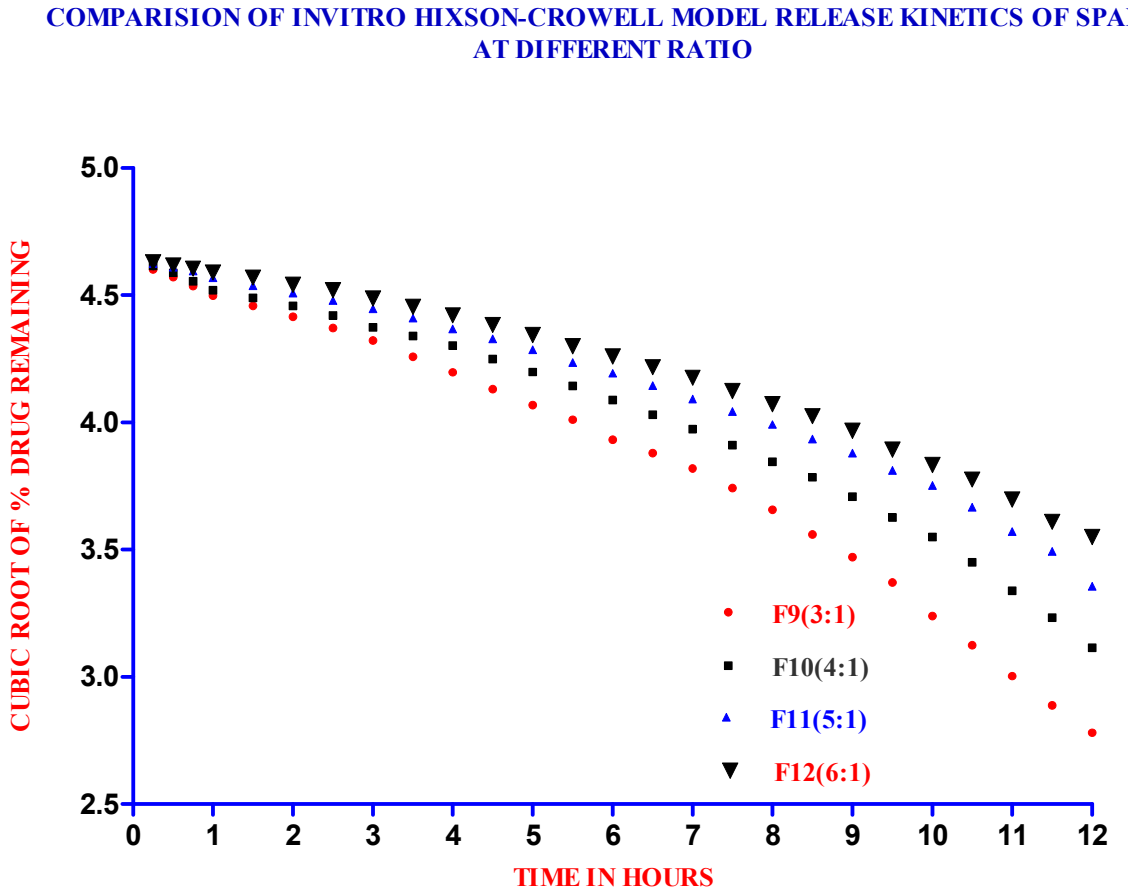
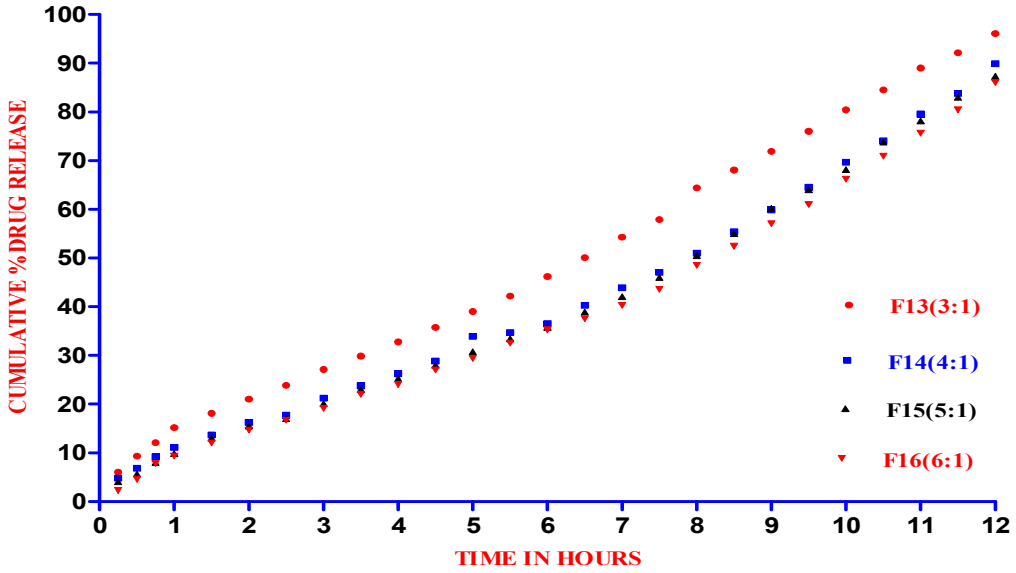
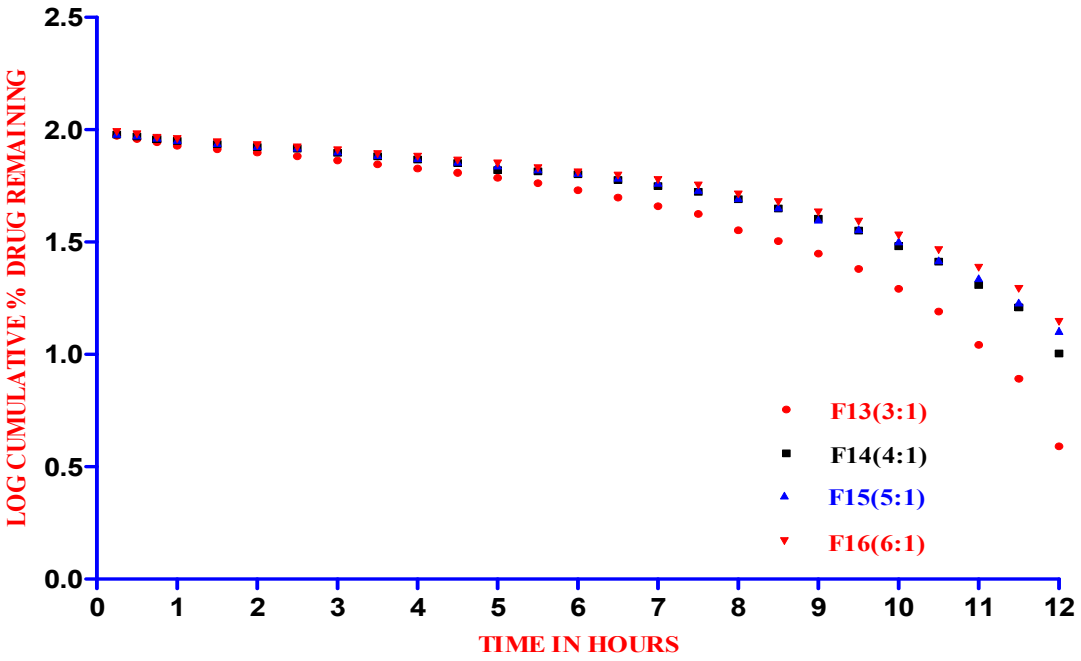


Figure 19c ***invitro*** **Release Kinetics of Niosomal Formulation Containing Span60 in Different Ratio**

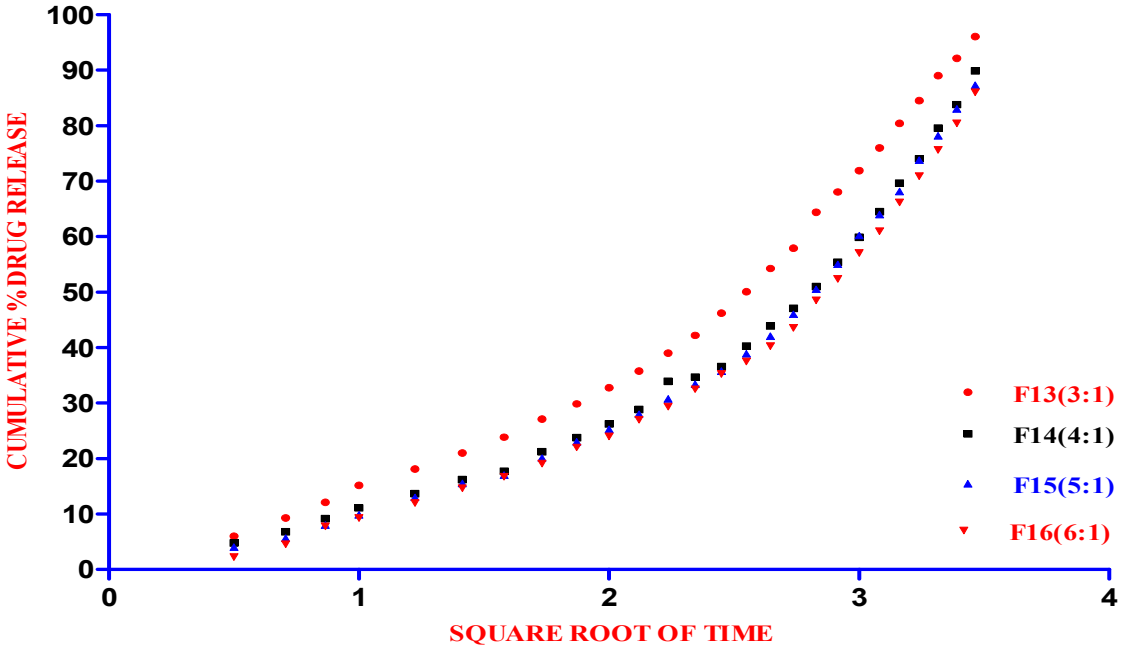
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF SPAN-80 AT DIFFERENT RATIO



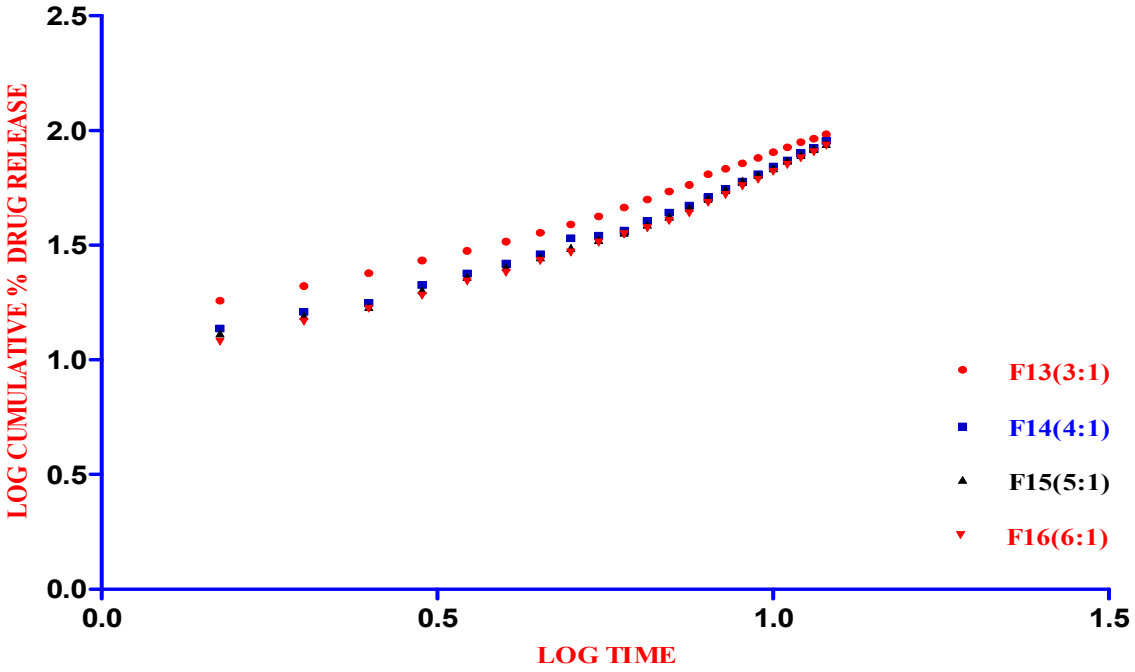
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF SPAN-80 AT DIFFERENT RATIO



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF SPAN-80 AT DIFFERENT RATIO



COMPARISION OF INVITRO KORSMEYER&PEPPAS MODEL RELEASE KINETICS OF SPAN-80 AT DIFFERENT RATIO



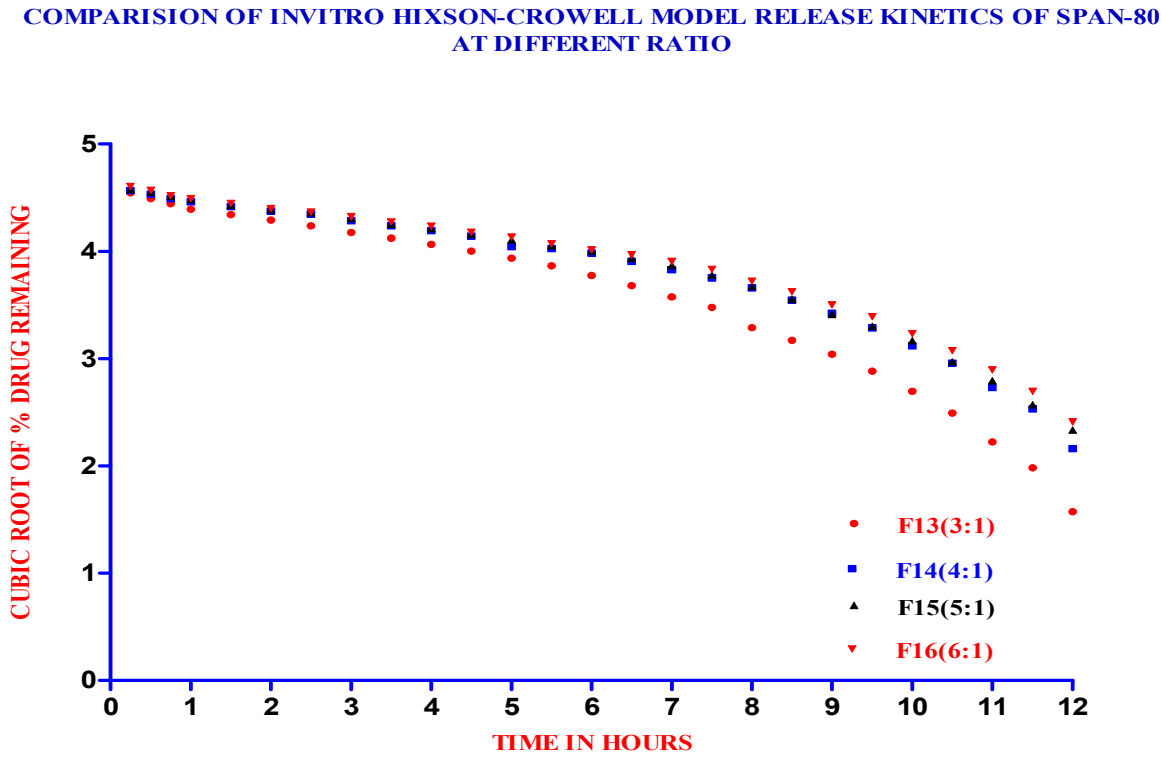
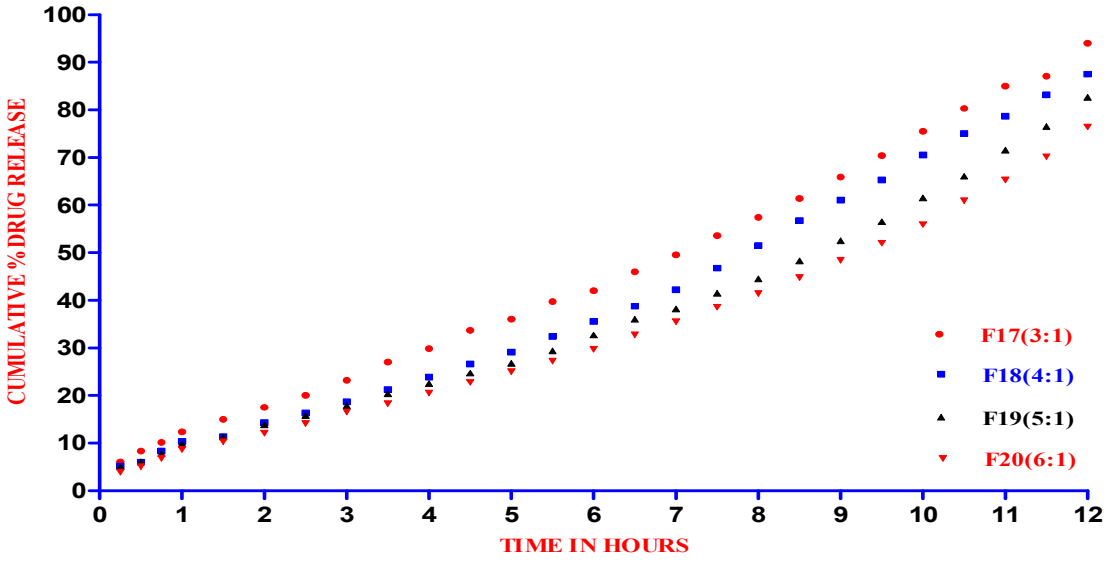
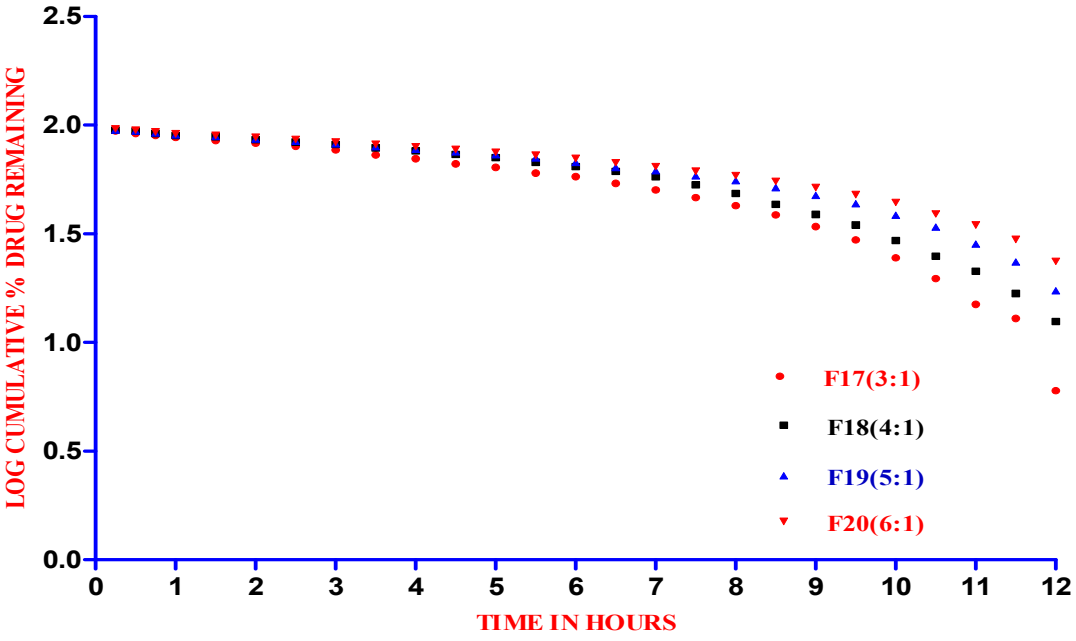


Figure 19d ***invitro*** **Release Kinetics of Niosomal Formulation Containing Span80 in Different Ratio**

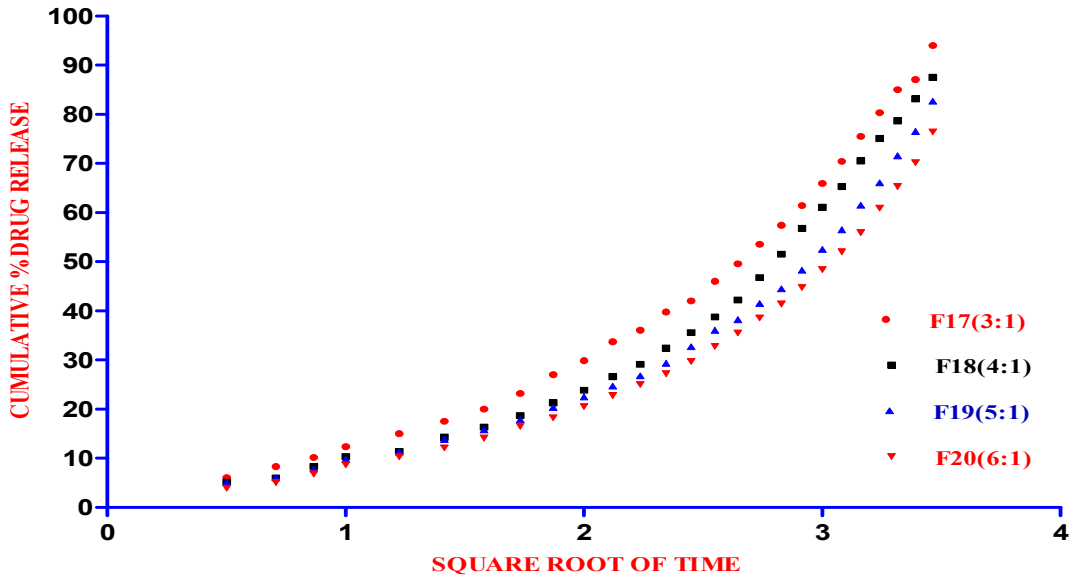
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF TWEEN-60 AT DIFFERENT RATIO



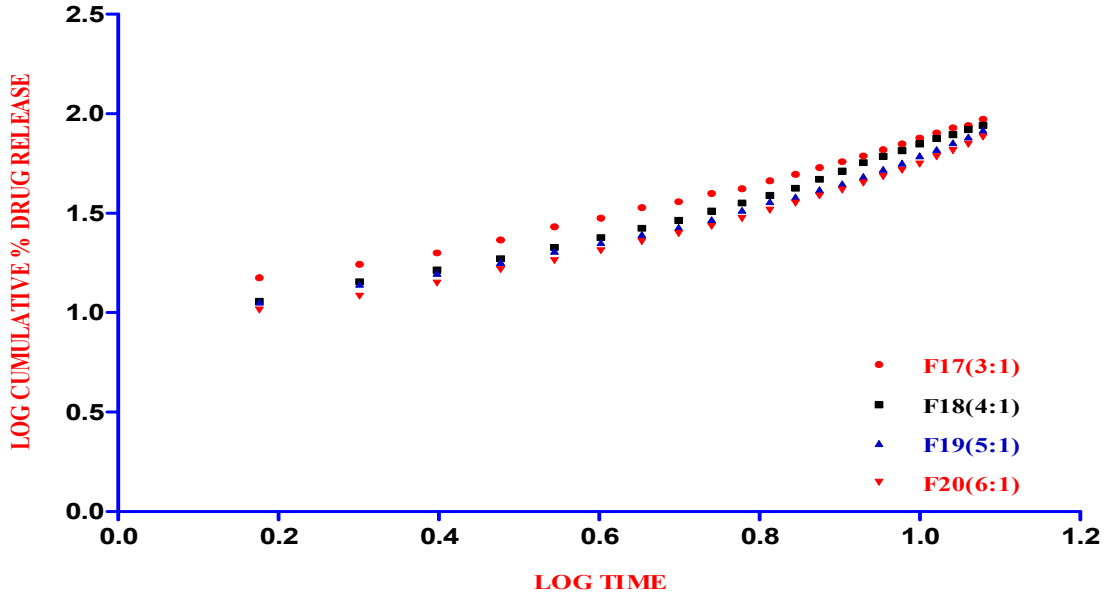
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF TWEEN-60 AT DIFFERENT RATIO



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF TWEEN-60 AT DIFFERENT RATIO



COMPARISION OF INVITRO KORSMEYER&PEPPAS MODEL RELEASE KINETICS OF TWEEN-60 AT DIFFERENT RATIO



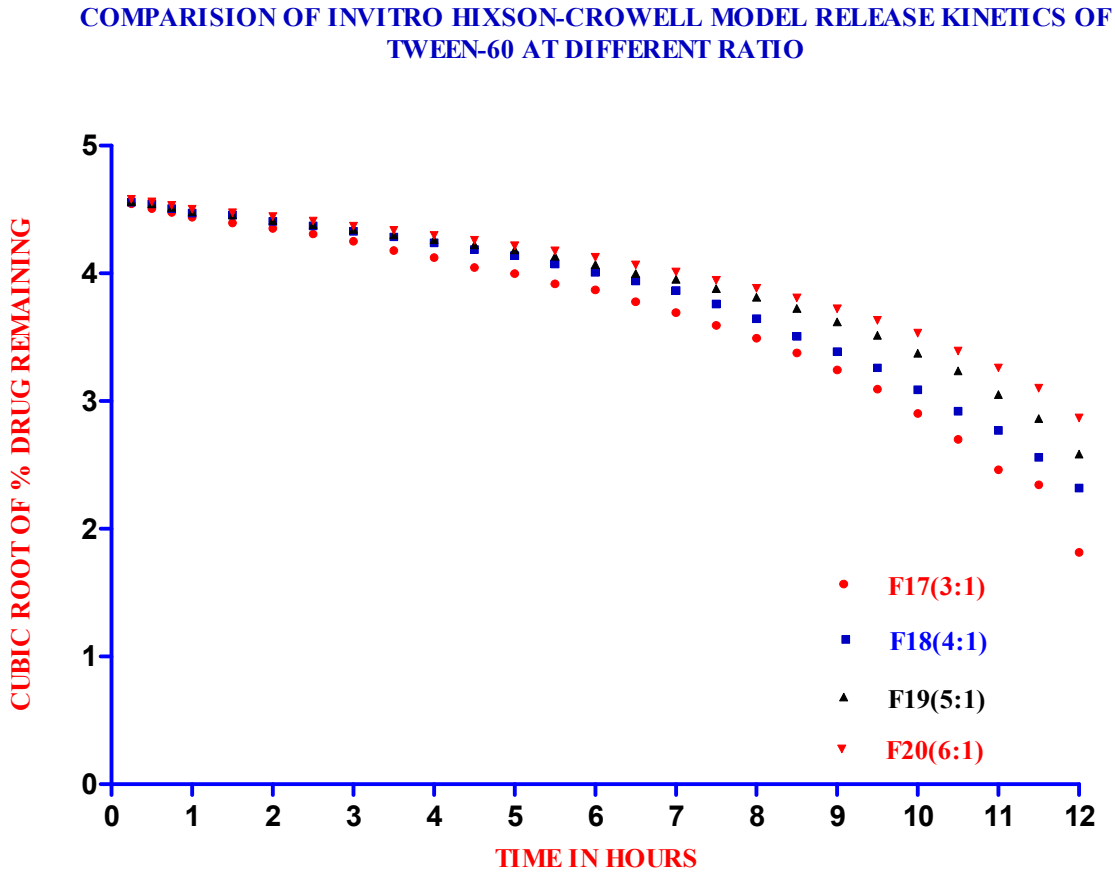
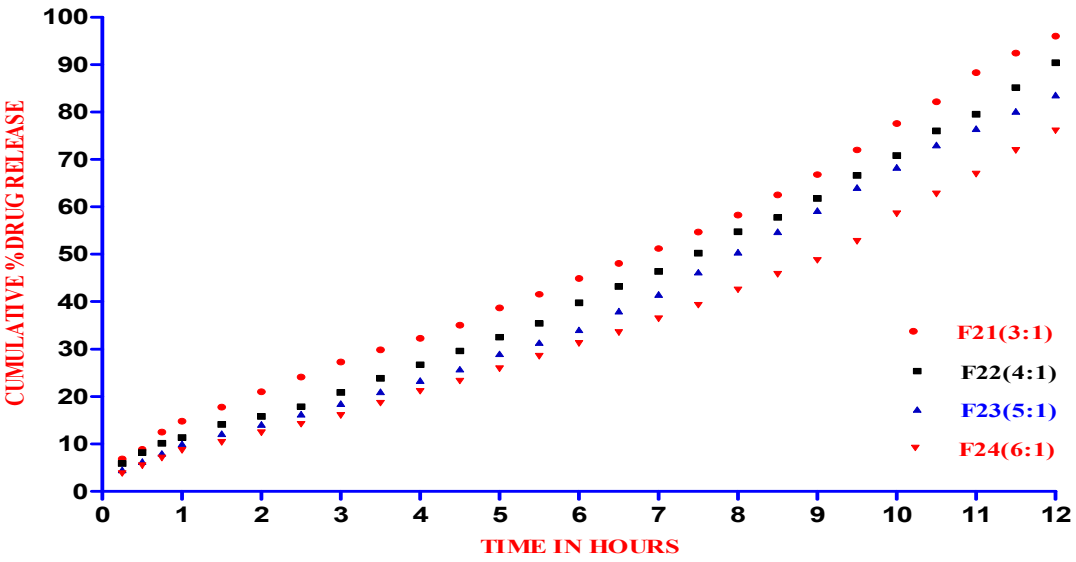
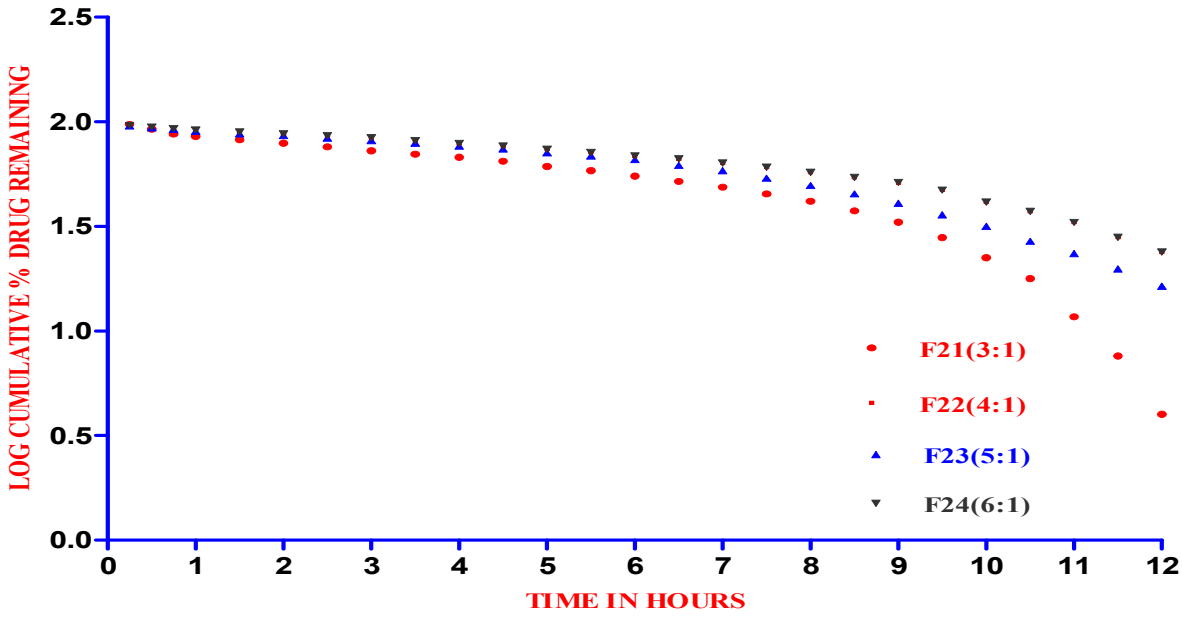


Figure 19e ***invitro*** **Release Kinetics of Niosomal Formulation Containing Tween60 in Different Ratio**

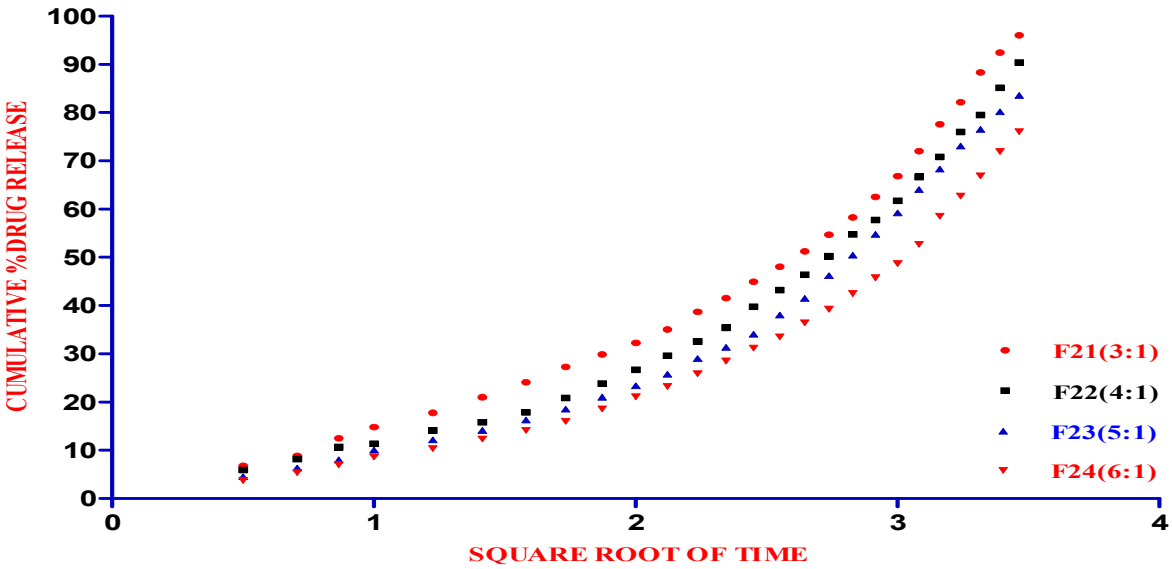
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF TWEEN-80 AT DIFFERENT RATIO



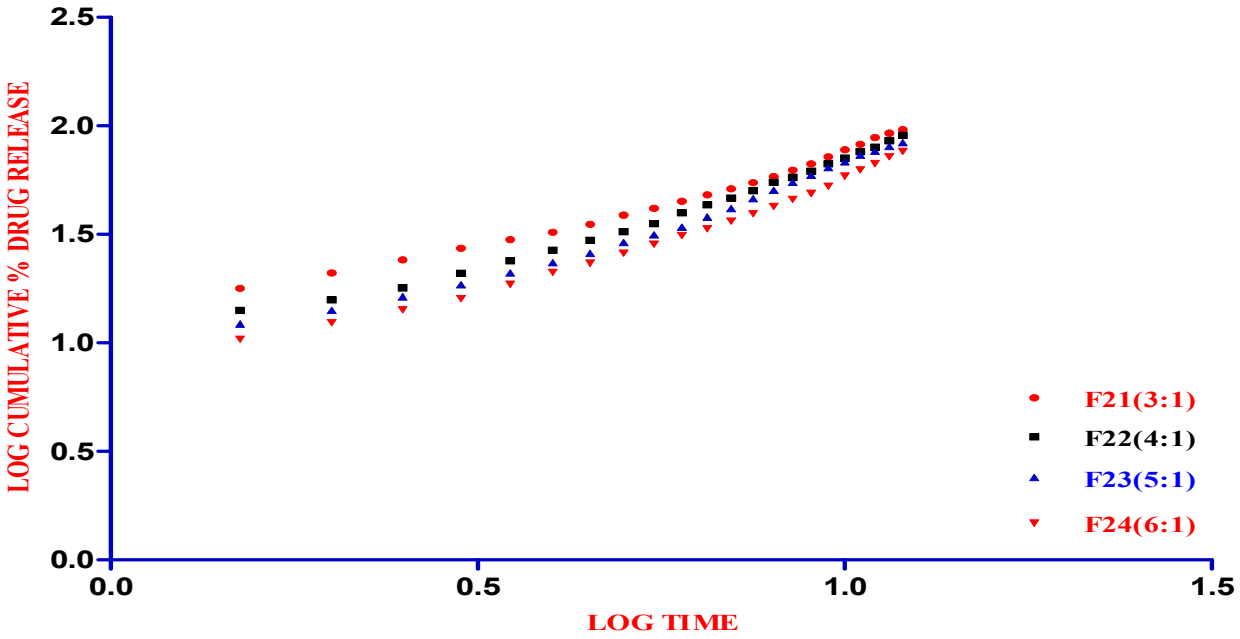
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF TWEEN-80 AT DIFFERENT RATIO



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF TWEEN-80 AT DIFFERENT RATIO



COMPARISION OF INVITRO KORSMEYER&PEPPAS MODEL RELEASE KINETICS OF TWEEN-80 AT DIFFERENT RATIO



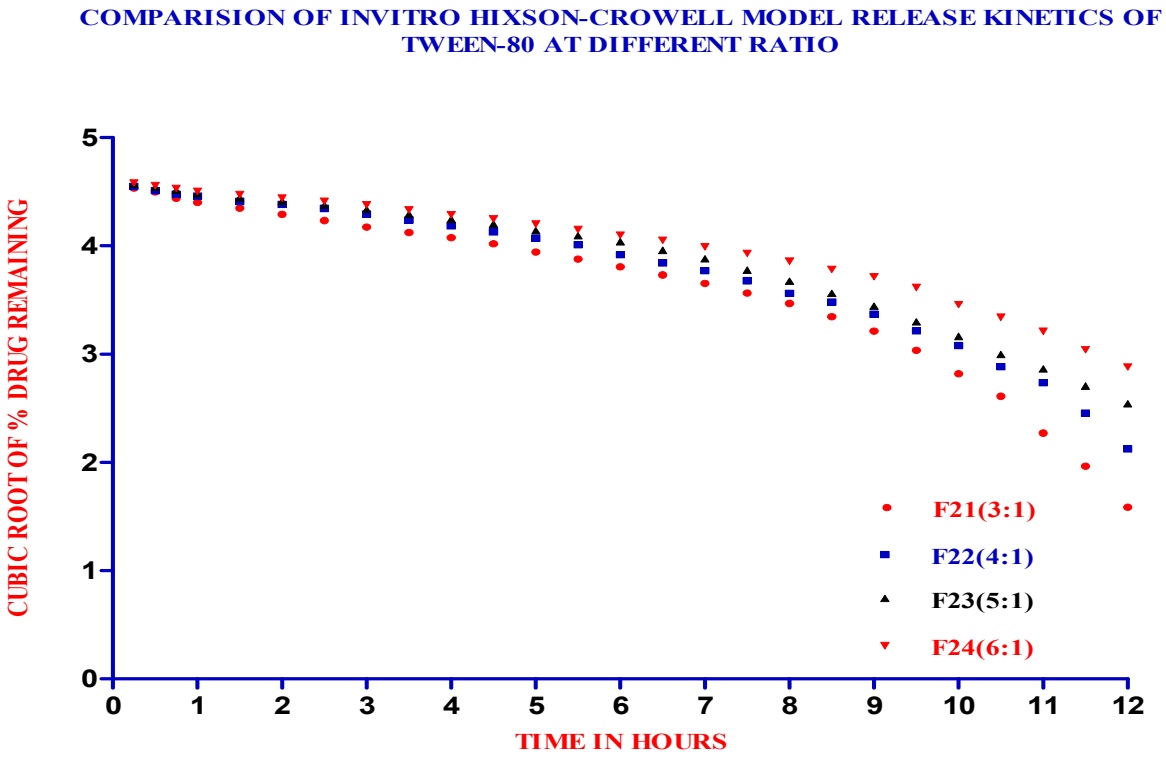
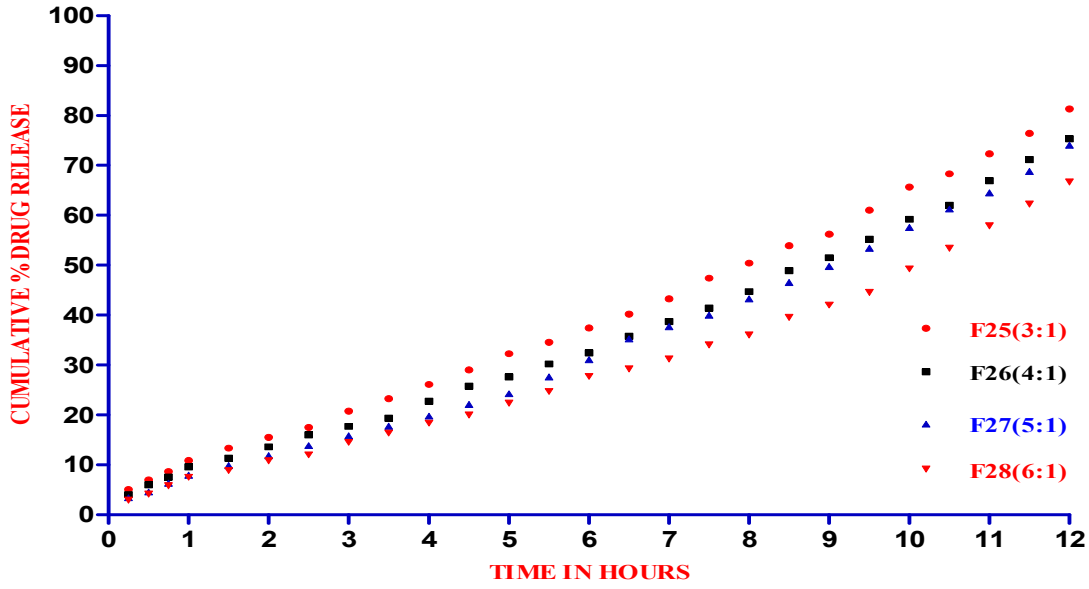
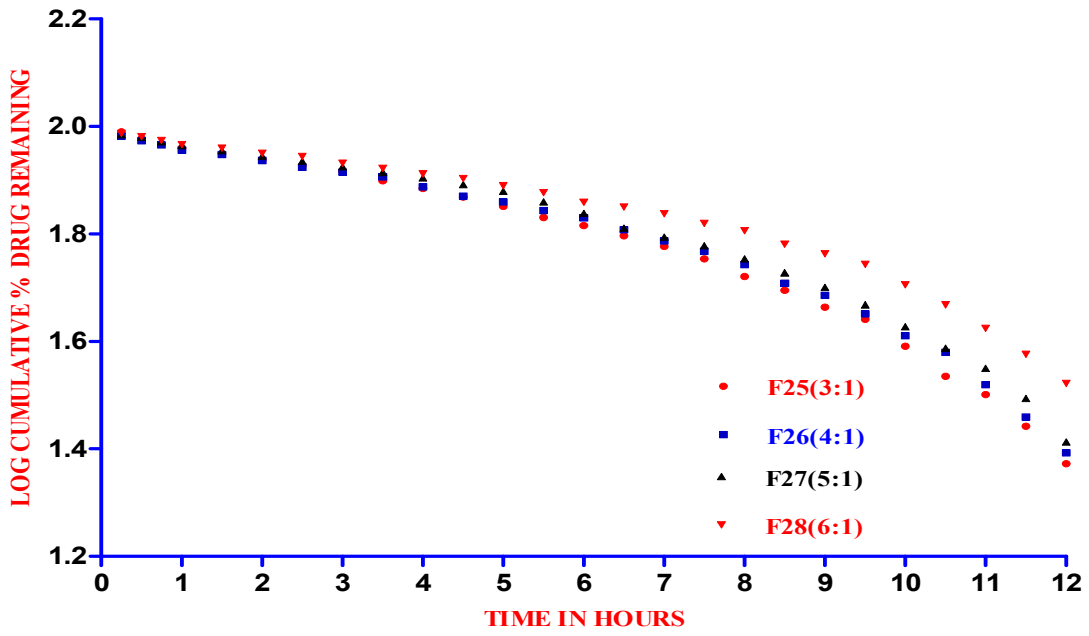


Figure 19f ***invitro*** **Release Kinetics of Niosomal Formulation Containing Tween80 in Different Ratio**

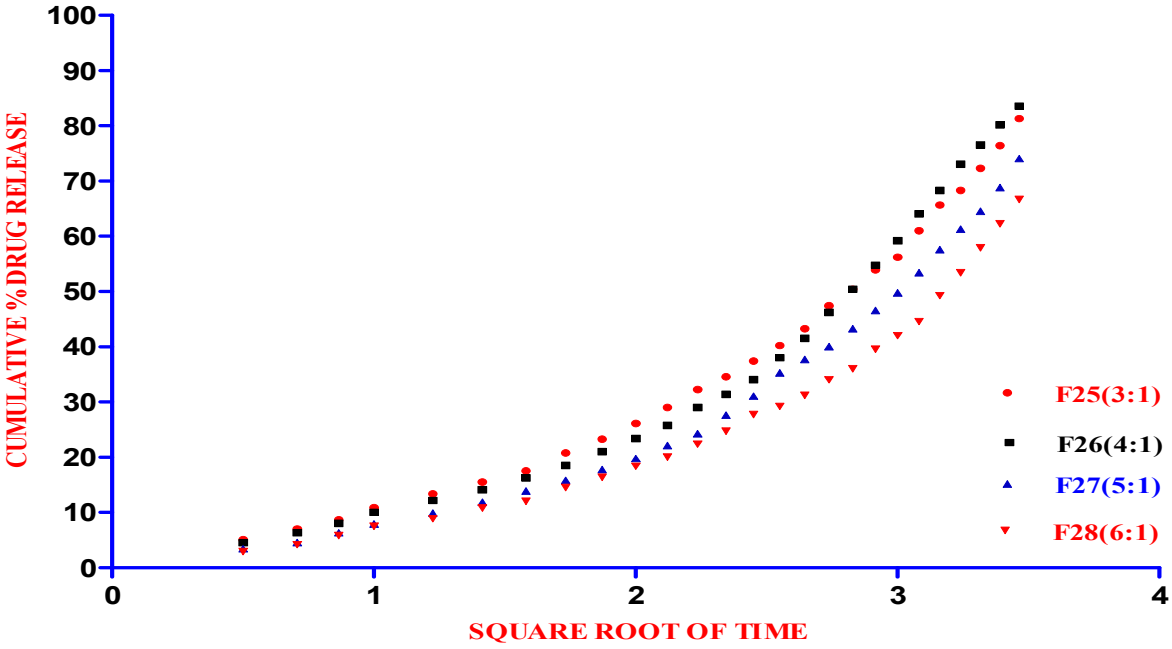
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF BRIJ-52 AT DIFFERENT RATIO



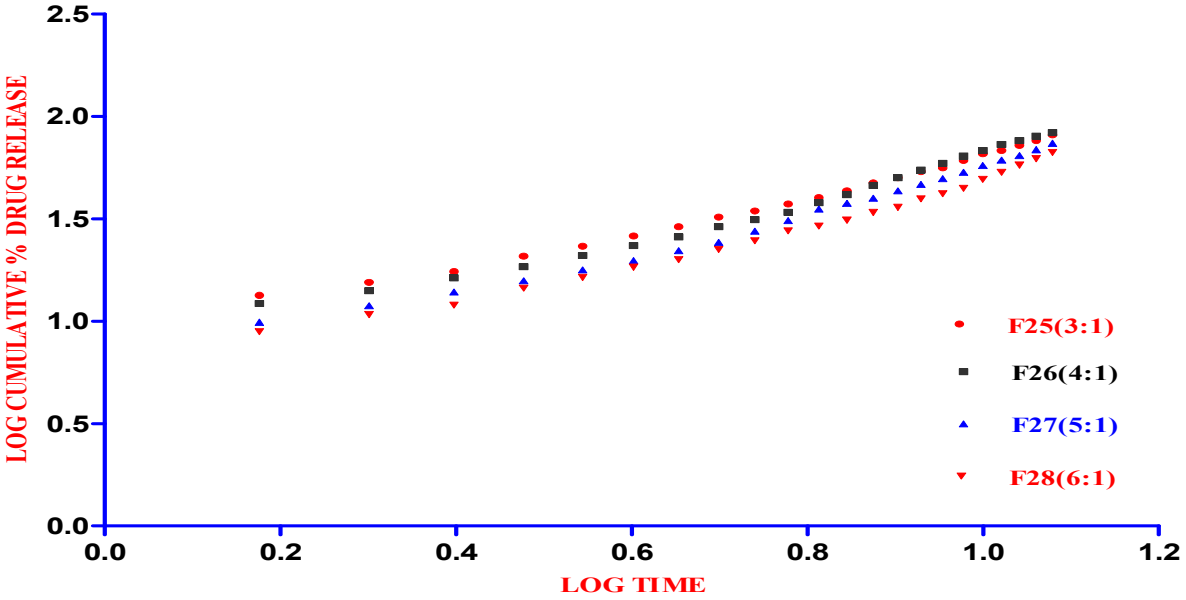
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF BRIJ-52 AT DIFFERENT RATIO



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF BRIJ-52 AT DIFFERENT RATIO



COMPARISION OF INVITRO KORSMEYER&PEPPAS MODEL RELEASE KINETICS OF BRIJ-52 AT DIFFERENT RATIO



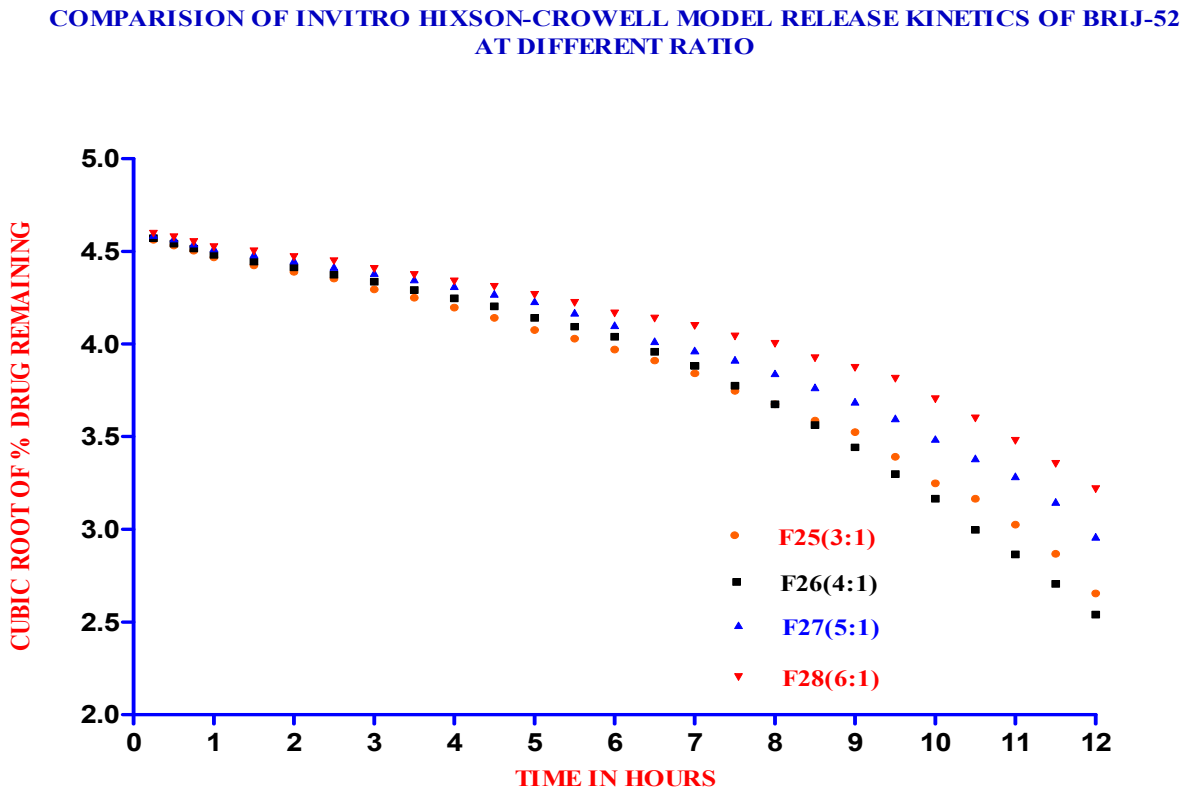
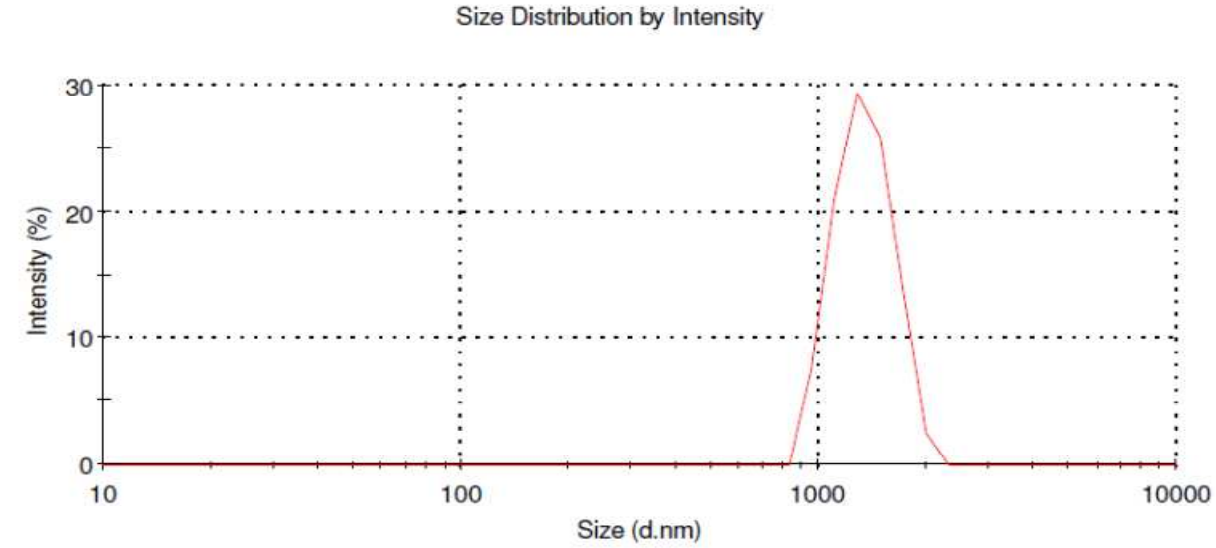


Figure 19g ***invitro*** **Release Kinetics of Niosomal Formulation Containing Brij-52 in Different Ratio**

20(a) FG12 (SPAN60 6:1)



20(b) FG21 (TWEEN80 3:1)

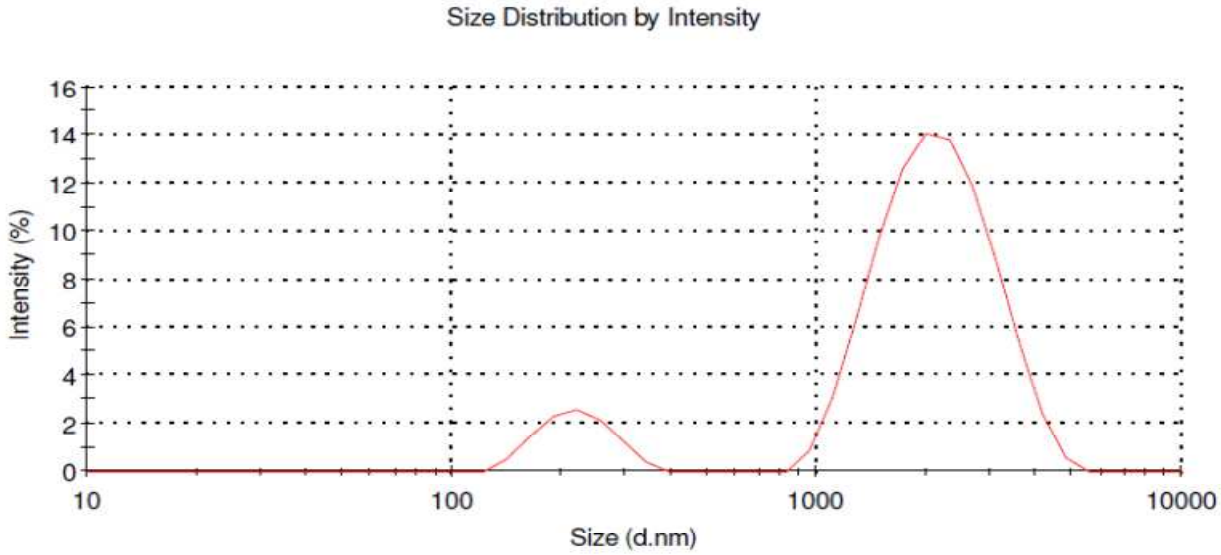
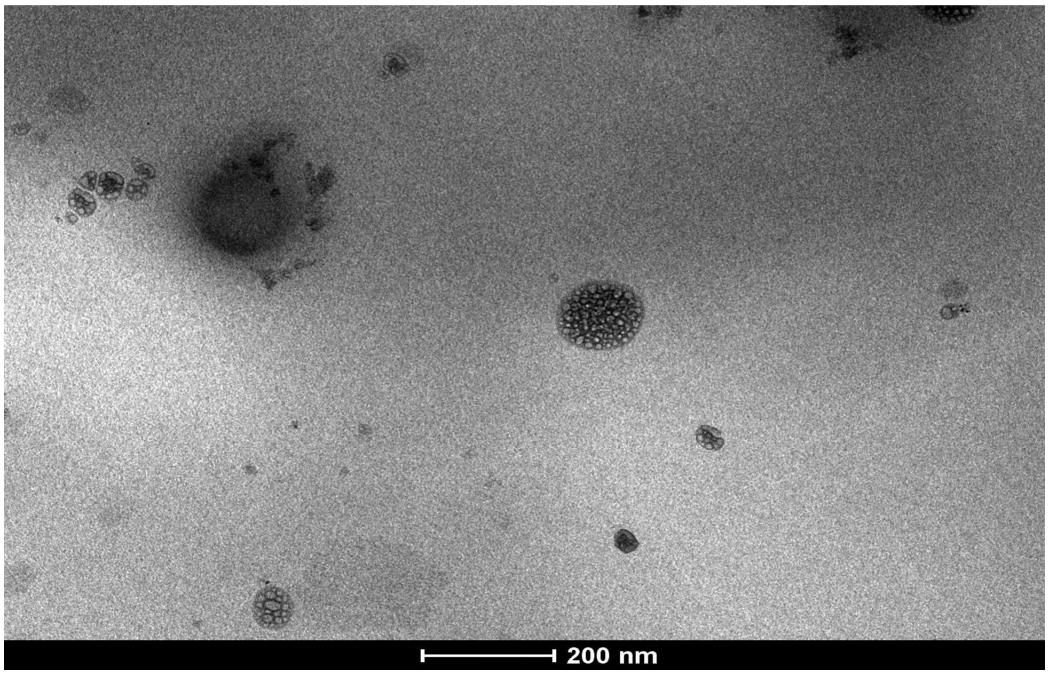


Figure 20 Particle Size Determination of Niosomal Gel Formulation Containing (A) Fg12 (Span60 6:1) (B) Fg21 (Tween80 3:1)

21a (a) FG12-(Span 60 6:1)



21a (b) FG12-(Span 60 6:1)

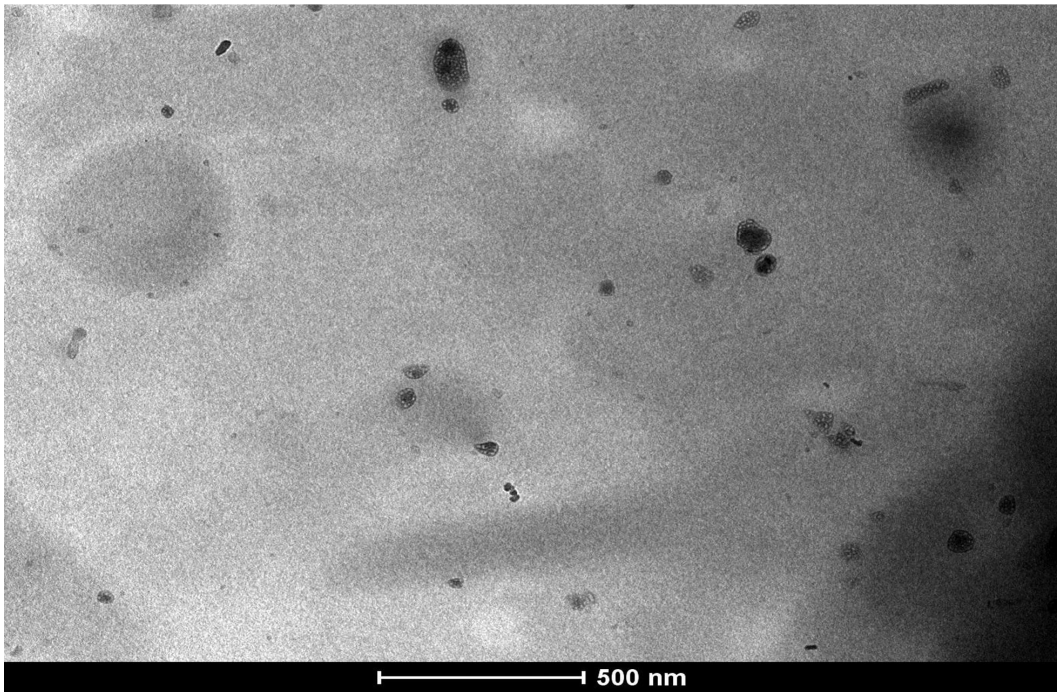
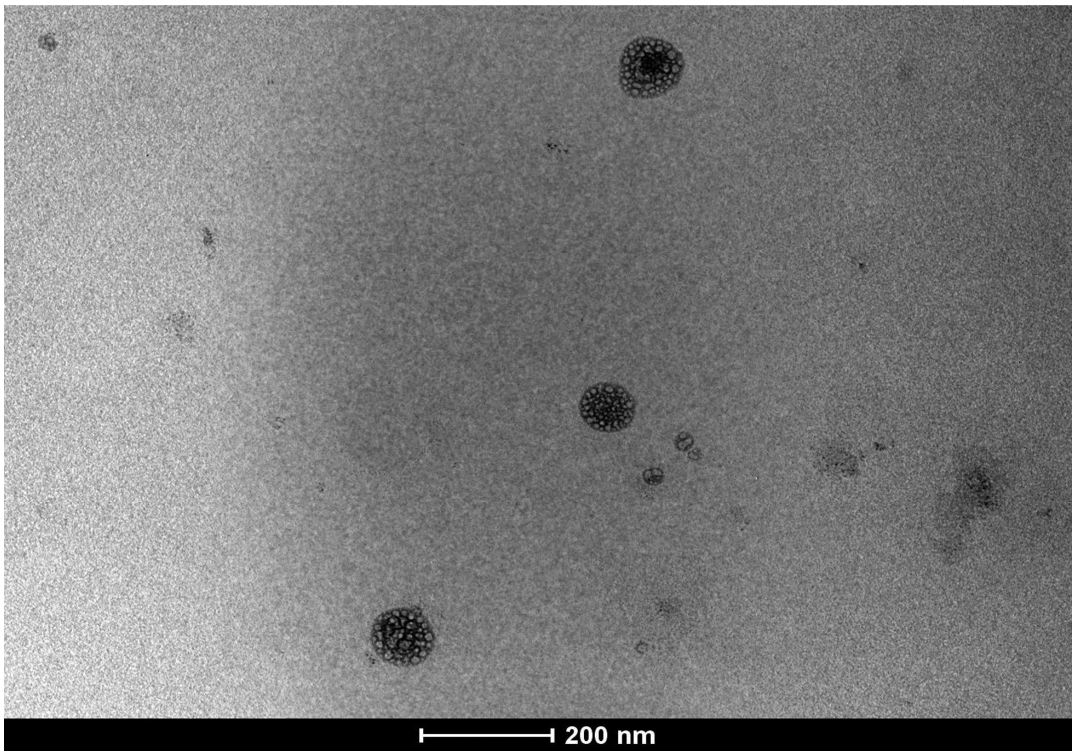


Figure 21 a Transmission Electron Microscopy Images of Niosomal Gel Containing (A) Fg12-(Span 60 6:1), (B) Fg12-(Span 60 6:1)

21b (a) FG21 (TWEEN 80 3:1)



21b (b) FG21 (TWEEN 80 3:1)

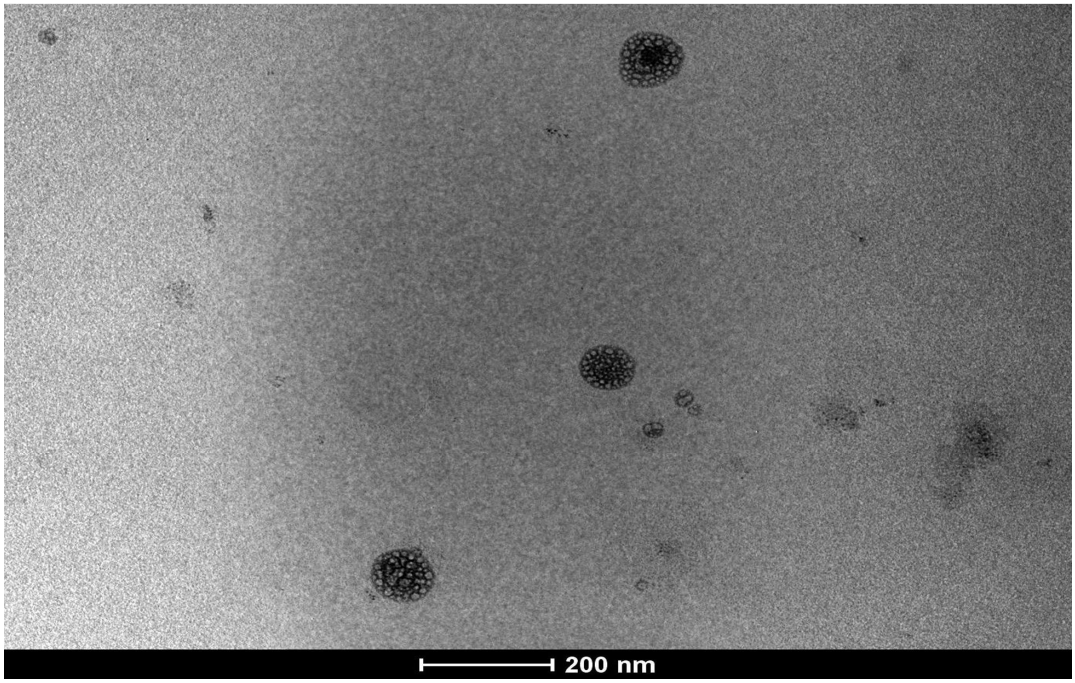


Figure 21 b Transmission Electron Microscopy Images Of Niosomal Gel Containing (A) Fg21 (Tween 80 3:1) 21b (B) Fg21 (Tween 80 3:1)

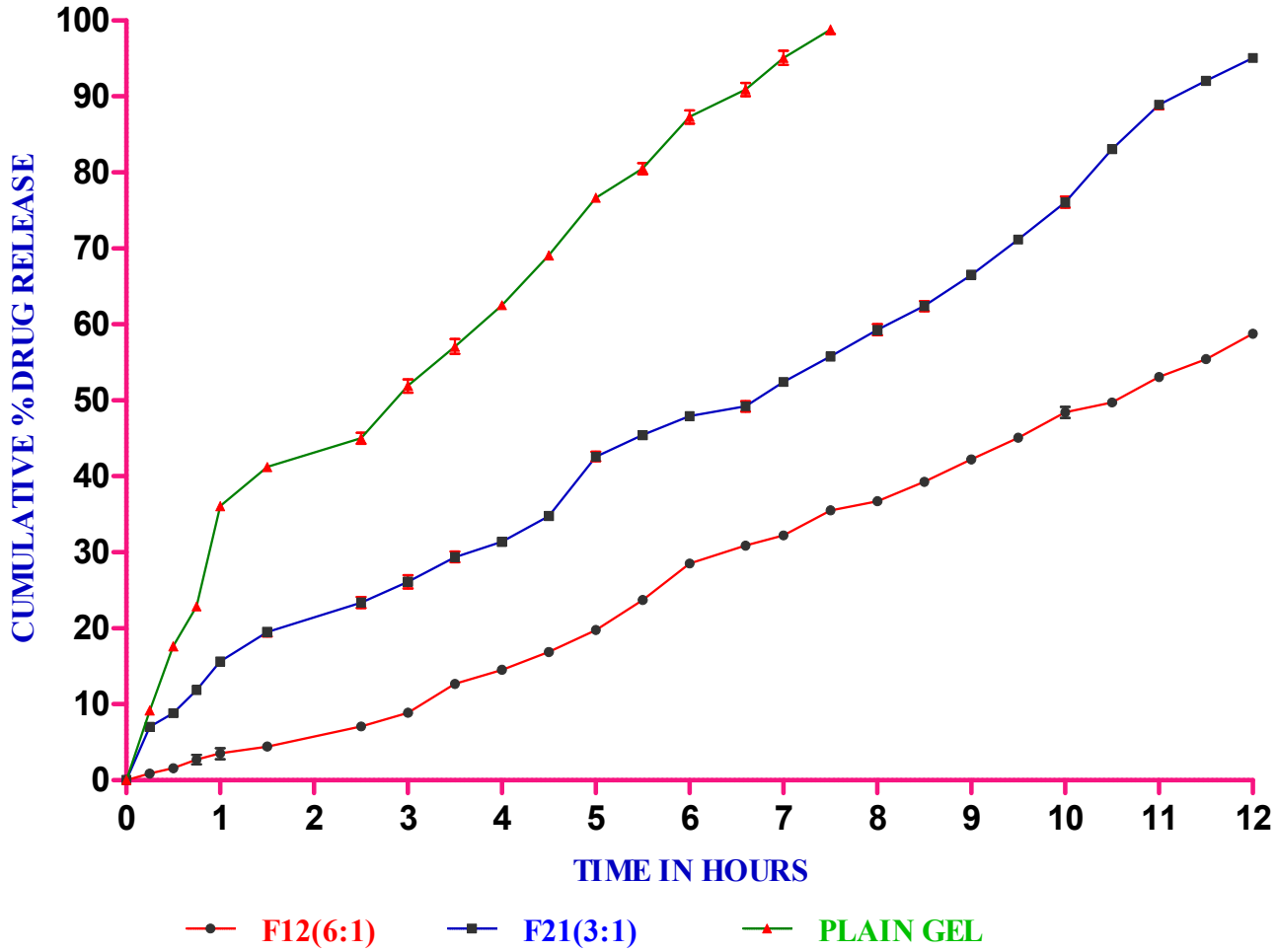
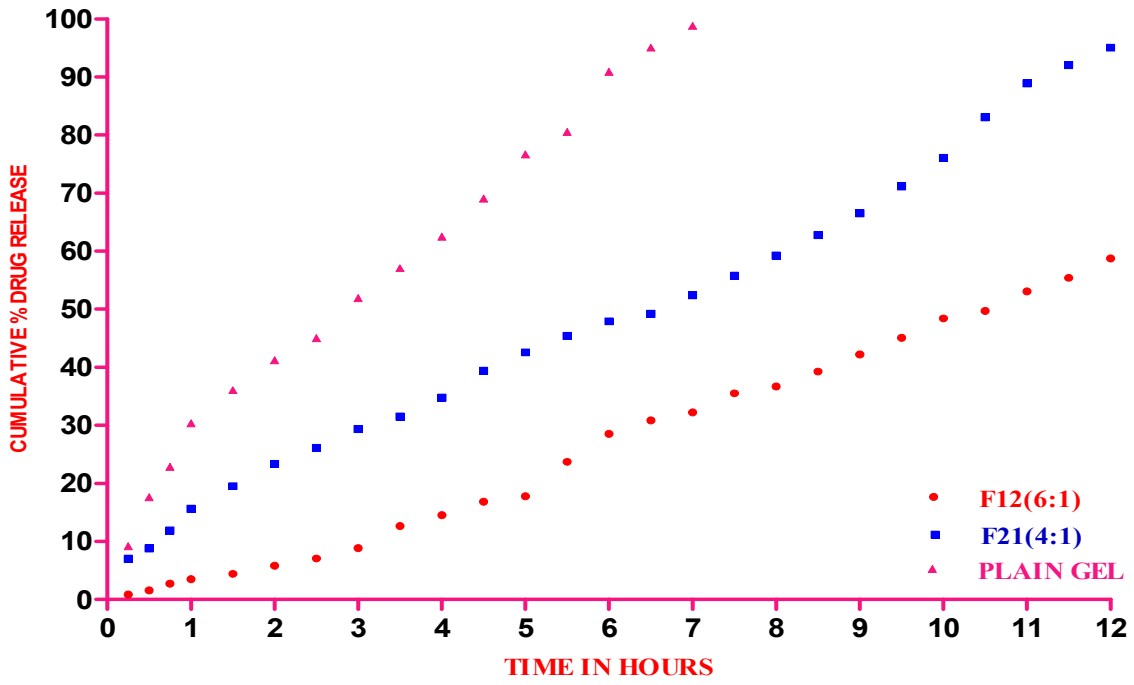
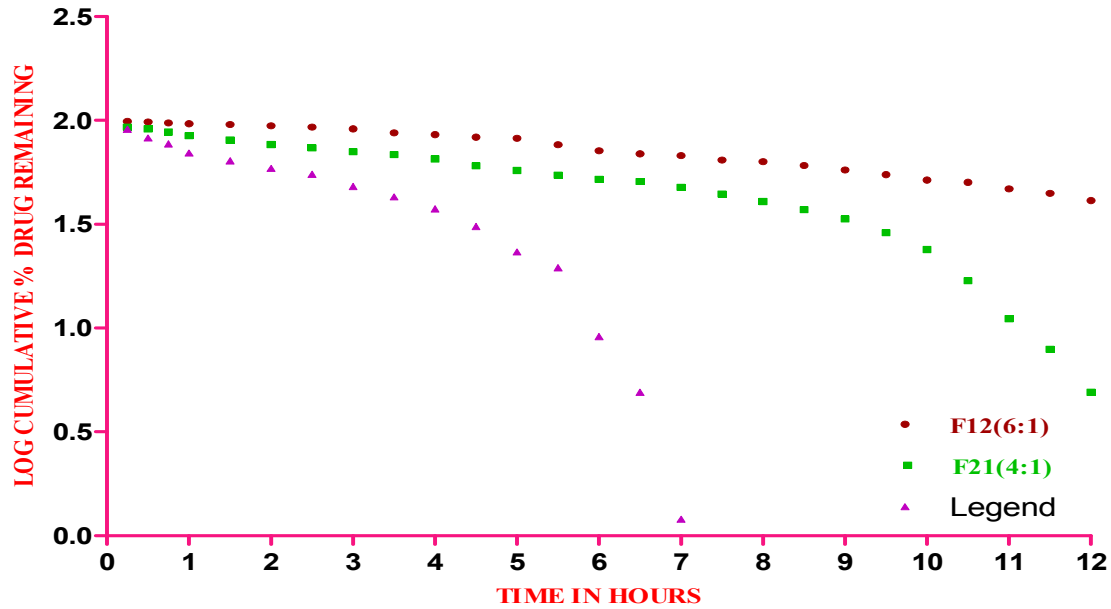


Figure 22 Comparison of *Invitro* Release of Plain Gel, Niosomel Gel with Highest and Lowest Entrapment Efficiency

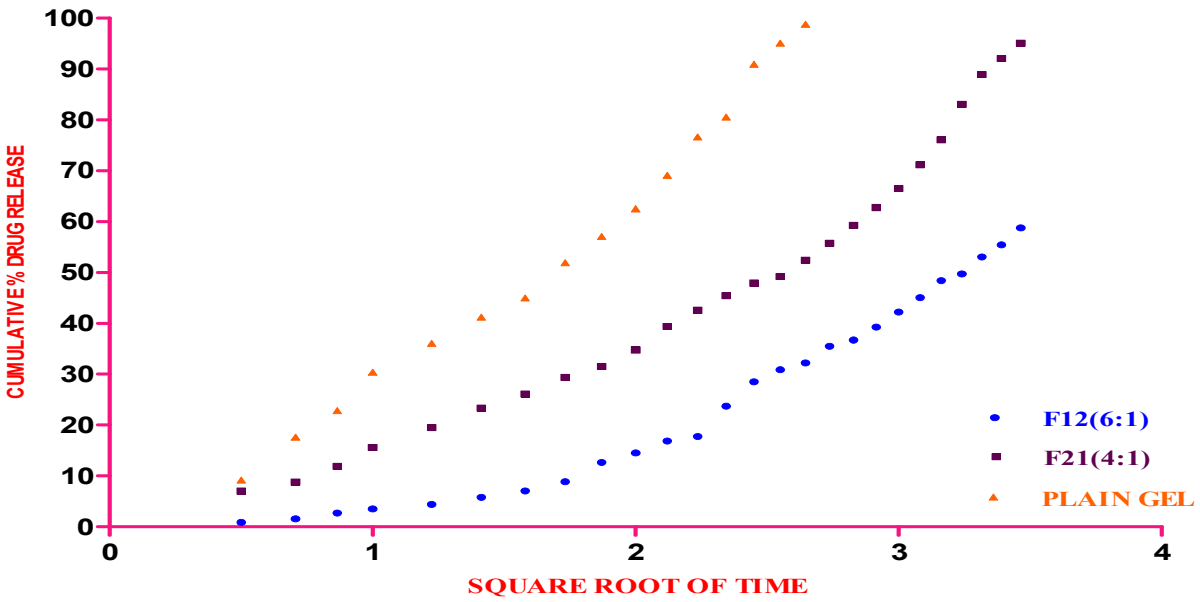
COMPARISION OF INVITRO ZERO ORDER RELEASE KINETICS OF NIOSOMAL GEL CONTAINING SPAN 60(6:1),TWEEN80(3:1) WITH PLAIN GEL



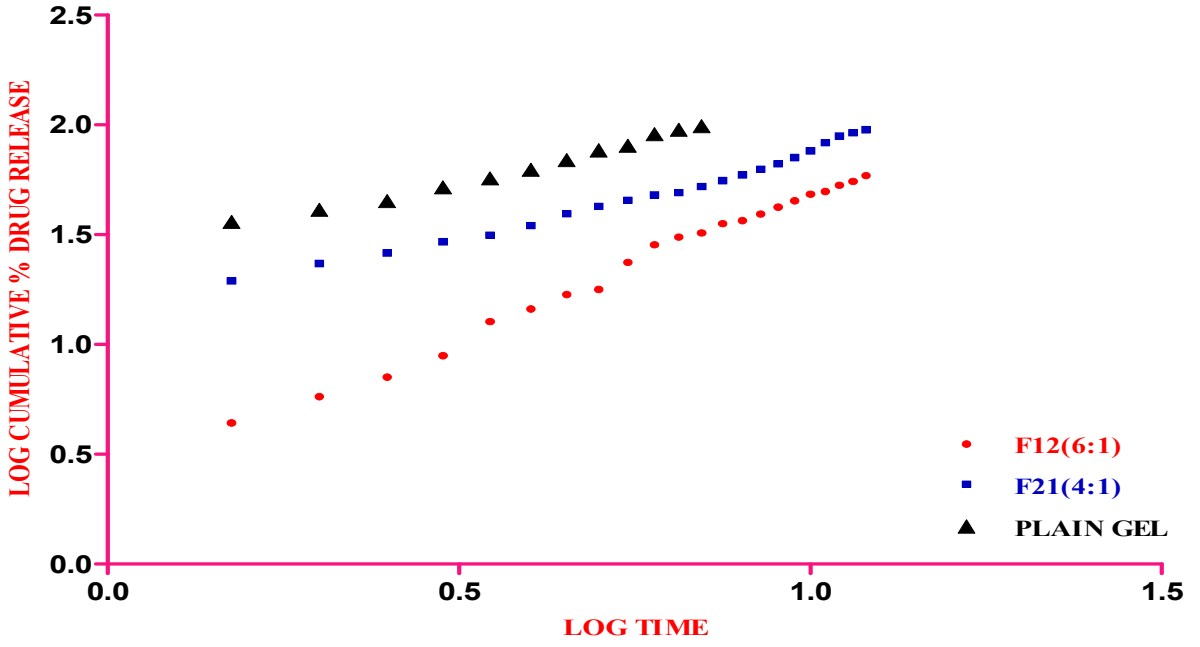
COMPARISION OF INVITRO FIRST ORDER RELEASE KINETICS OF NIOSOMAL GEL CONTAINING SPAN 60(6:1),TWEEN80(3:1) WITH PLAIN GEL



COMPARISION OF INVITRO HIGUCHI MODEL RELEASE KINETICS OF NIOSOMAL GEL CONTAINNING SPAN 60(6:1),TWEEN80(3:1) WITH PLAIN GEL



COMPARISION OF INVITRO KORSMEYER &PEPPAS MODEL RELEASE KINETICS OF NIOSOMAL GEL CONTAINING SPAN 60(6:1),TWEEN80(3:1) WITH PLAIN GEL



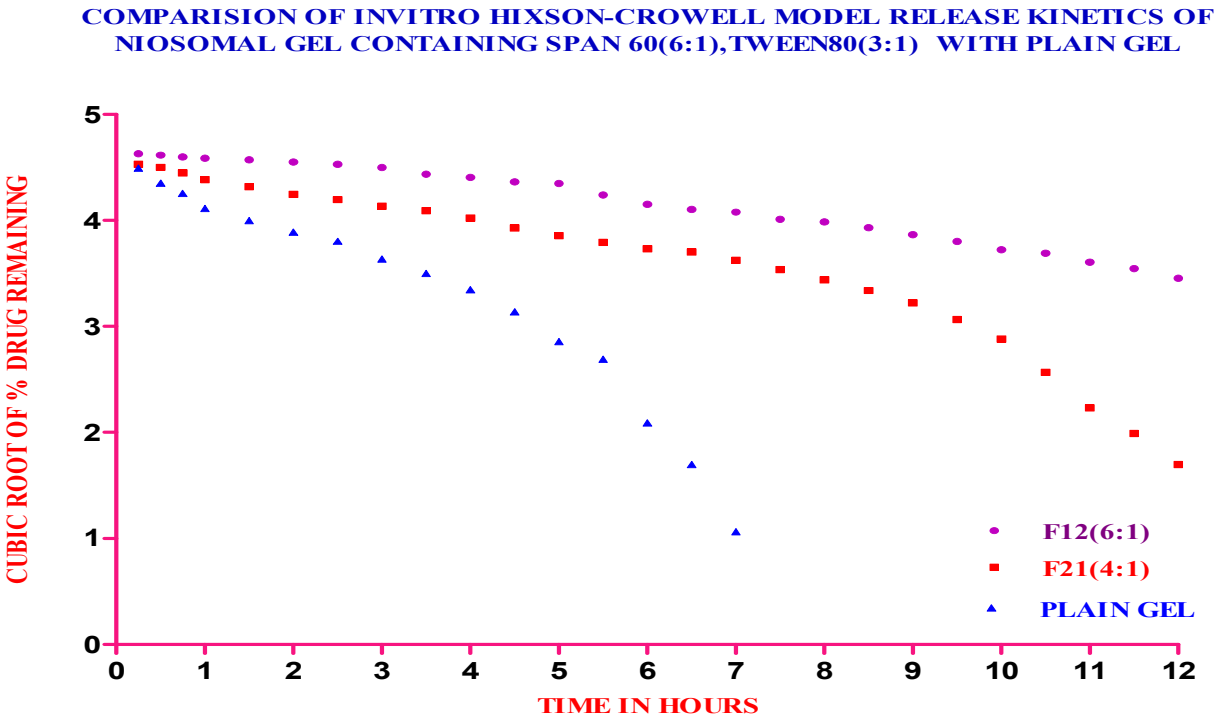


Figure 23 ***invitro*** **Release Kinetics of Niosomal Gel Containing F12 (6:1), F214:1) in Different Ratio**

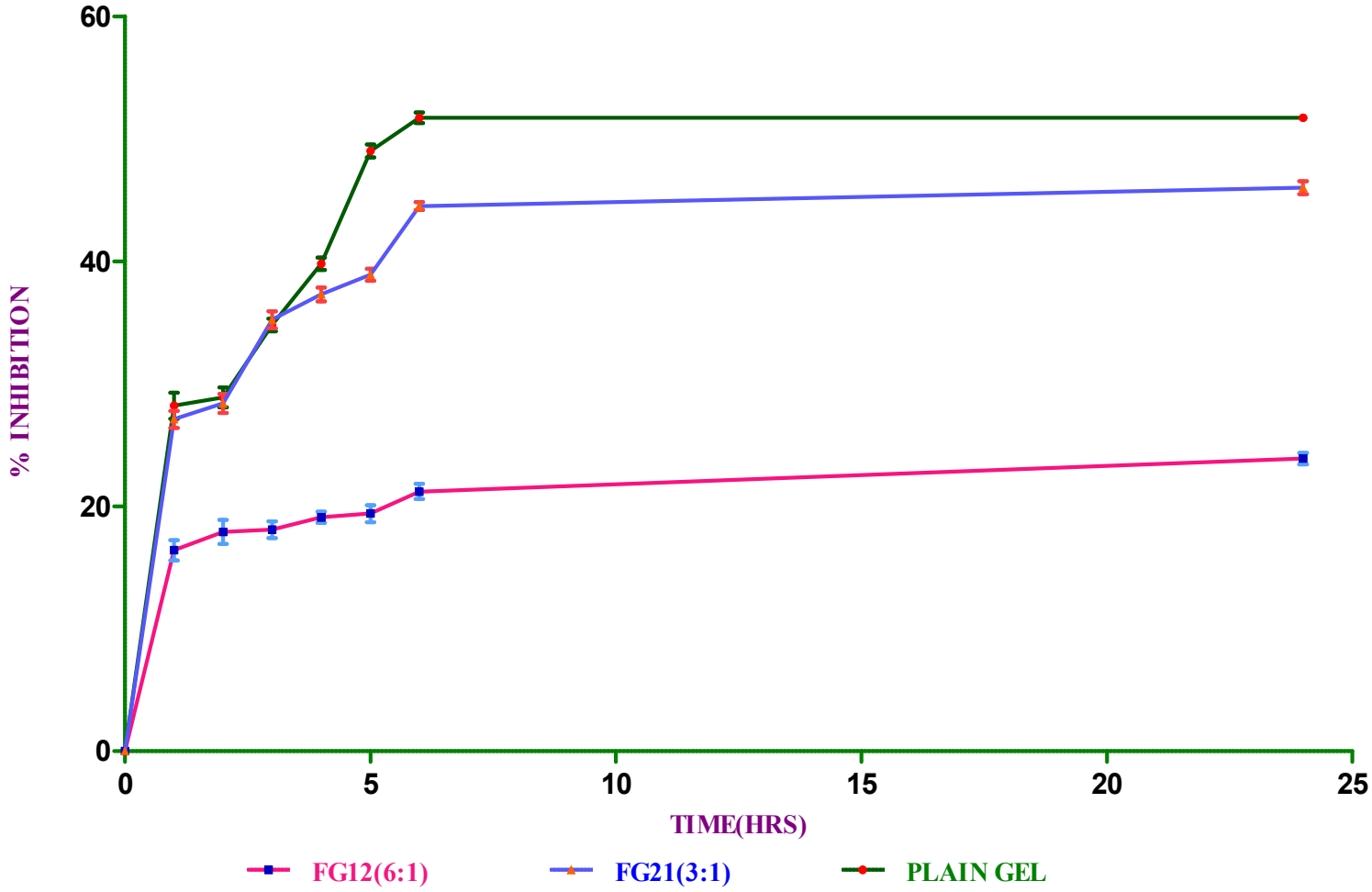


Figure 24 Comparison of Percentage Inhibition of Rat Hind Paw Edema Method using Plain Etoricoxib Gel and Niosomal Gel Containing FG12 and FG21

CHAPTER-XIII

SUMMARY AND CONCLUSION

- The niosomal formulations were successfully prepared by thin film hydration technique using cholesterol and Span20, 40, 60, 80 Tween 60, 80 and Brij-52 as non-ionic surfactant. The presence of cholesterol made the niosomes more stable with high entrapment efficiency and retention properties. The highest entrapment efficiency was observed with span60 and it may be concluded that the entrapment efficiency may be improved using surfactant with decrease HLB value and highest phase transition temperature.
- FT-IR and DSC investigations confirmed that there was no interaction of cholesterol and surfactant towards the drug.
- Brook field viscometer confirmed the Pseudoplastic behaviour of prepared niosomal gel.
- TEM image showed spherical vesicles
- Malvern zeta sizer used to confirmed the vesicle size of niosomal dispersion
- Release kinetics showed sustained release and anomalous diffusion indicated that niosomes of etoricoxib can deliver significant levels of drug.
- The invitro release and invivo anti-inflammatory studies of niosomal gel as compared to plain gel indicated that the prolonged drug release behavior with sustained therapeutic action.
- The result indicates that the niosomal gel can be used as novel drug delivery carrier for skin targeting of etoricoxib for its anti-inflammatory action.

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